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Article

# Discovery of CJ-2360 as a Potent and Orally Active Inhibitor of Anaplastic Lymphoma Kinase Capable of Achieving Complete Tumor Regression

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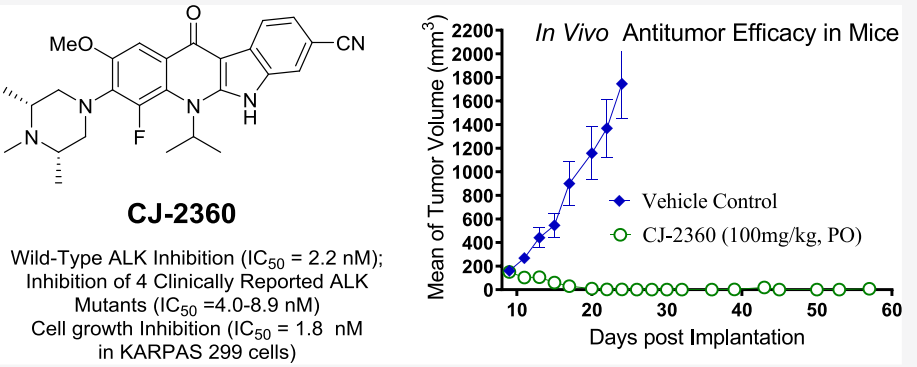


**ABSTRACT:** We report herein the discovery of a class of potent small-molecule inhibitors of anaplastic lymphoma kinase (ALK) containing a fused indoloquinoline scaffold. The most promising compound CJ-2360 has an $IC_{50}$ value of 2.2 nM against wild-type ALK and low-nanomolar potency against several clinically reported ALK mutants. This compound is capable of achieving complete tumor regression in the ALK-positive KARPAS-299 xenograft model with oral administration in mice. CJ-2360 represents a promising ALK inhibitor for advanced preclinical development.

## INTRODUCTION

Anaplastic lymphoma kinase (ALK) is a receptor tyrosine kinase belonging to the insulin receptor (IR) kinase superfamily. ALK is expressed primarily in adult brain tissue and plays an important role in the development of the nervous system.[1]

Deregulation of ALK was originally discovered by the identification of a t(2;5) chromosomal translocation in anaplastic large cell non-Hodgkin's lymphoma (ALCL).[2] The nucleophosmin (NPM)-ALK fusion protein produced by this translocation results in constitutive activation of the ALK kinase.[3] Subsequently, fusion of ALK with other genes has been identified in ALCL,[4] inflammatory myofibroblastic tumor,[5] diffuse large B-cell lymphoma,[6] squamous cell carcinoma,[7] and non-small-cell lung cancer.[8−10] In addition to chromosomal translocations leading to fusion proteins, amplification of the ALK gene and activating point mutations of the wild-type ALK protein have been reported in neuroblastoma,[11−13] ovarian cancer,[14] and patients with inflammatory breast cancer.[15] Accordingly, ALK has been pursued as an attractive therapeutic target for treatment of various blood and solid tumors containing an ALK fusion (ALK-positive tumors).

Extensive efforts have been devoted in the last decade to the discovery and development of ALK inhibitors.[16−23] Crizotinib (**1**)[24,25] was the first ALK inhibitor approved by the U.S. FDA in 2011 as a first-line treatment for ALK-positive lung cancer patients. Subsequently, ceritinib (**2**, LDK378),[26] alectinib (**3**, CH5424802),[27,28] and brigatinib (**4**, AP26113)[29] were approved by the U.S. FDA for treatment of patients with advanced (metastatic), ALK-positive non-small-cell lung cancer (NSCLC), whose disease has deteriorated after treatment, or who could not tolerate treatment with crizotinib (Figure 1). Recently, lorlatinib[30] (**5**, PF-6463922) received accelerated approval by the U.S. FDA for patients with ALK-positive NSCLC, whose metastatic disease has progressed on crizotinib and at least one other ALK inhibitor or whose disease has

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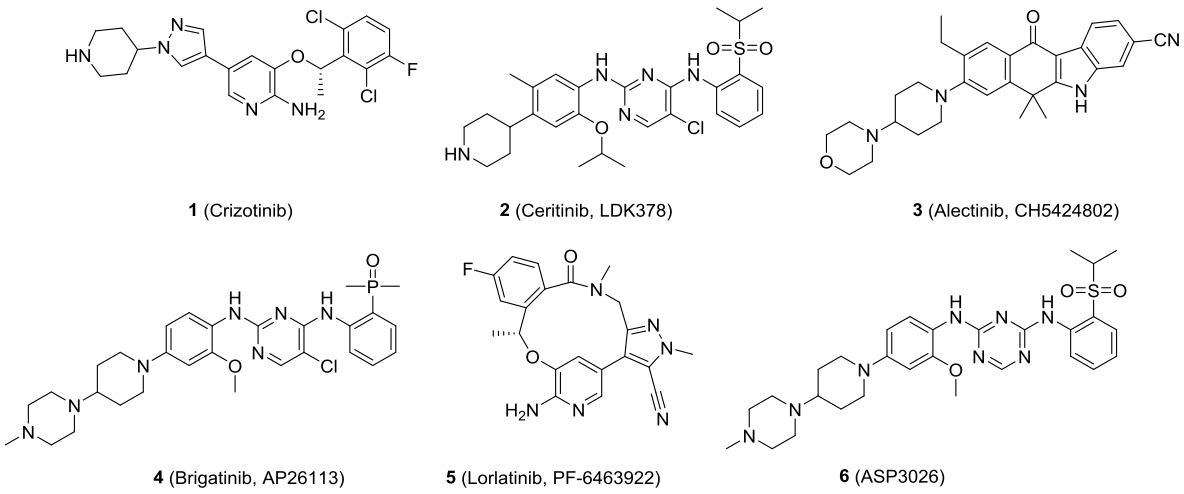
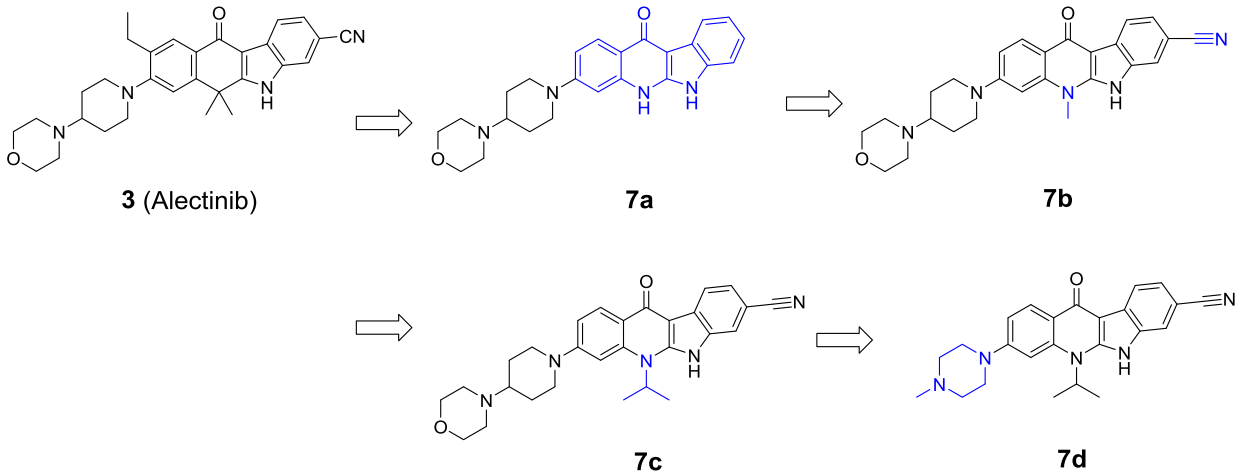



**Figure 1.** Chemical structures of ALK inhibitors approved or currently in clinical trials.

**Table 1. Design of Indoloquinolones as Potential ALK Inhibitors, Chemical Structures, and Their *In Vitro* ALK Inhibitory Activity**




| compound | $IC_{50}$(nM) in inhibition of ALK enzymatic activity |
|---|---|
| **7a** | >50,000 |
| **7b** | 2503 ± 545 |
| **7c** | 28.6 ± 12.6 |
| **7d** | 21.3 ± 7.4 |

progressed on alectinib or ceritinib as the first ALK inhibitor therapy for metastatic disease. Additional ALK inhibitors, including ASP3026 (**6**),[31] are currently in clinical development.

In the present study, we report the discovery of a new class of potent and selective ALK inhibitors, exemplified by CJ-2360 (**11n**). CJ-2360 (**11n**) potently inhibits wild-type ALK kinase and several clinically reported ALK mutants and is capable of achieving complete tumor regression in the ALK-positive KARPAS-299 xenograft model with oral administration.

## RESULTS AND DISCUSSION

Among all the ALK inhibitors that have been approved or are currently in clinical trials, alectinib (**3**) has a unique, fused tetracyclic system and has been shown to be highly selective against ALK over other kinases.[27] We selected alectinib (**3**) as the template for the design of a new class of ALK inhibitors.

We proposed that the indoloquinolone structure could mimic the tetracyclic core in alectinib and, to test this idea, we synthesized compounds **7a** and **7b** using the same soluble group as in alectinib (Table 1). While **7a** shows no inhibitory activity in our *in vitro* ALK enzymatic assay at concentrations up to 50 $\mu$M, **7b** displays an $IC_{50}$ value of 2.5 $\mu$M (Table 1). Compound **7b** therefore represented a reasonable starting point for further optimization.

In our modeled structure of **7b** in complex with ALK, there is additional room available around the *N*-methyl group in **7b** (Supporting Information). We explored this site by changing the *N*-methyl group in **7b** to *N*-isopropyl, yielding **7c** (Table 1). Compound **7c** exhibits an $IC_{50}$ value of 28.6 nM in ALK inhibition (Table 1) and is nearly 100 times more potent than **7b**.

The 4-(piperidin-4-yl)morpholinyl soluble group in alectinib was shown to be the primary metabolic site.[32] Accordingly, we replaced the 4-(piperidin-4-yl)morpholinyl group in **7c** with 1-methyl-piperazinyl and obtained **7d** (Table 1). Compound **7d** has an $IC_{50}$ value of 21.3 nM in ALK inhibition and is thus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equipotent with **7c** (Table 1). We employed **7c** as our template compound for further modifications.

We developed the binding model of **7d** in complex with ALK based on the cocrystal alectinib (CH5424802) complexed with ALK (PDB ID: 3AOX,[27] Figure 2A) to guide our further

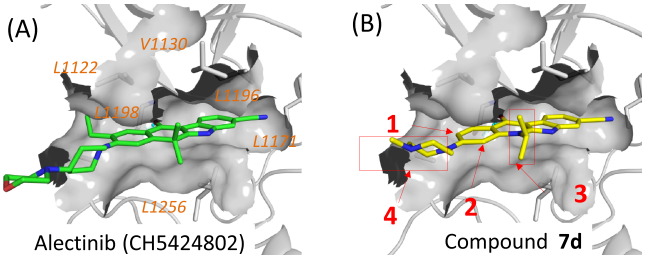



**Figure 2.** (A) Previously reported cocrystal structure of alectinib (CH5424802) in complex with ALK (PDB ID: 3AOX). (B) Modeled structure of compound **7d** in complex with ALK. Four sites in **7d** identified for optimization are labeled with red arrows.

optimization efforts. The predicted binding model for **7d** shows that the tetracyclic core structure in **7d** mimics the tetracyclic core in alectinib, its cyano group interacts with a deep hydrophobic pocket formed by the L1196, L1171, and V1180 residues in ALK, and the carbonyl group of **7d** forms a hydrogen bond with the backbone amide group of L1198. Furthermore, the isopropyl group of **7d** occupies the same space as the dimethyl group in CH5424802.

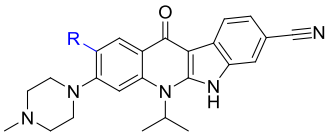
Compound **7d** lacks the corresponding ethyl group in alectinib, which in the cocrystal structure (Figure 2A), has hydrophobic interactions with L1198, A1200, and L1122 residues in ALK. We thus performed modifications on the phenyl ring in **7d** to explore the interactions with L1198, A1200, and L1122 residues, obtaining the results summarized in Table 2.

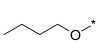
Installation of an ethyl group on the phenyl ring in **7d** yielded compound **8b**, which has an $IC_{50}$ value of 2.1 nM in inhibition of ALK and is therefore 10 times more potent than **7d**. Changing the ethyl group in **8b** to *n*-propyl resulted in **8c**, which has an $IC_{50}$ value of 1.0 nM in ALK inhibition and is thus 2 times more potent than **8b**. Replacing the ethyl group in **8b** with a bromine atom generated **8a**, which is slightly more potent than **8b**. Installation of an isobutyl group at the same site resulted in **8d**, which has an $IC_{50}$ value of 2.5 nM and is thus equipotent with **8b**. Interestingly, replacing the isobutyl group in **8d** with an isopropyl group, which led to **8e**, reduces the ALK inhibitory activity by a factor of 16.

We next synthesized compounds **8f**–**8i** by introducing a series of alkoxy groups at this site. Compounds **8f** with a methoxyl group, **8g** with a propoxyl group, **8h** with a butoxyl group, and **8i** with an isopropoxyl group all have potent and similar ALK inhibitory activity with $IC_{50}$ values of 3.7–5.8 nM.

We evaluated these potent ALK inhibitors in Table 2 for their cell growth inhibitory activity in the KARPAS-299 cell line carrying an ALK fusion protein (ALK-positive), obtaining the results in Table 2. Compound **7d** has an $IC_{50}$ value of 514.6 nM. In comparison, compounds **8a**–**8d**, which have an improved ALK inhibitory activity over **7d**, have $IC_{50}$ values of 24.2–36.8 nM and are >10 times more potent than **7d**. Although **8e** is 2 times less potent than **7d** in ALK inhibition, **8e** is 4 times more potent than **7d** in inhibition of KARPAS-299 cell growth, suggesting enhanced cell permeability.

**Table 2. Structure–Activity Relationship Studies of Phenyl Substitutions on ALK Enzymatic Inhibition and Cell Growth Inhibitory Activity in the ALK-Positive KARPAS-299 Cell Line**




| compd | R | $IC_{50}$ (nM) (ALK Inhibition) | $IC_{50}$ (nM) (Cell Growth Inhibition in KARPAS-299) |
|---|---|---|---|
| **7d** | H | 21.3±7.4 | 514.6 |
| **8a** | Br | 1.3±0.2 | 29.5 |
| **8b** | Et | 2.1±1.2 | 36.8 |
| **8c** | *n*-Pr | 1.0±0.1 | 24.2 |
| **8d** |  | 2.5±1.1 | 24.3 |
| **8e** |  | 40.5±14.6 | 122.5 |
| **8f** |  | 4.8±1.0 | 206.7 |
| **8g** |  | 3.7±0.6 | 73.1 |
| **8h** |  | 5.4±0.4 | 61.0 |
| **8i** |  | 5.8±1.2 | 140.2 |

Next, we installed one or two fluorine atoms onto the phenyl ring in **7d** to examine the effect on ALK inhibition and cell growth inhibition in the KARPAS-299 cell line and obtained the results summarized in Table 3. Installation of a single fluorine atom onto the phenyl ring of **7d** gave **9a**, whose ALK inhibitory activity is similar to that of **7d**. However, the cellular activity of **9a** in KARPAS-299 is improved by 10 times over that of **7d**. Compound **9b** containing two fluorine substitutions in the phenyl ring of **7d** is 3 times more potent than **9a** in ALK inhibition but has a similar cell growth inhibitory activity in KARPAS-299 when compared to **9a**. Compound **9c**, which was obtained by introduction of a fluorine atom into **8f**, is 2 times less potent than **8f** in ALK inhibition but is 10 times more potent than **8f** in cell growth inhibition in the KARPAS-299 cell line. These data indicated that installation of one or two fluorine atoms on the phenyl ring significantly improves the cellular potency of the resulting compounds.

Next, we explored the effect of different substitutions on the quinolone nitrogen in **8f** and obtained the results summarized in Table 4.

Replacement of the isopropyl group in **8f** by an ethyl or *n*-propyl group yielded **10a** and **10b**, respectively. While **10a** and **10b** are only 2–5 times less potent than **8f** in ALK inhibition, they are much less potent than **8f** in cell growth inhibition in the KARPAS-299 cell line. In fact, **10a** with an ethyl group has a minimal cell growth inhibitory activity at concentrations up to 100 $\mu$M. Replacement of the isopropyl group in **8f** with an isobutyl or isopentyl group yielded **10c** and **10d**, respectively. Compounds **10c** and **10d** display very similar ALK inhibition and cell growth inhibition activity compared to **8f**. Replacing the isopropyl group in **8f** with a cyclopropyl, cyclobutyl, cyclopentyl, or cyclohexyl group resulted in **10e**, **10f**, **10g**, and **10h**, respectively. Compound **10e** is 3 times less potent in ALK inhibition than **8f** but is >30 times less potent than **8f** in


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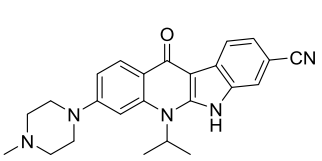
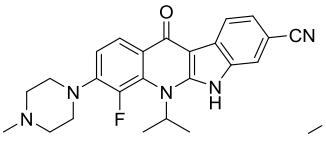
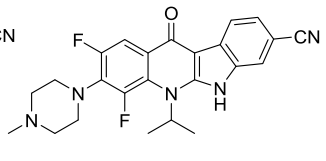
**Table 3. Effect of Fluorine Substitution on the Phenyl Ring**



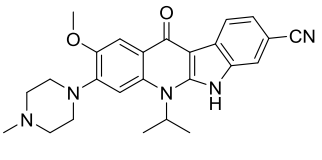
**7d**



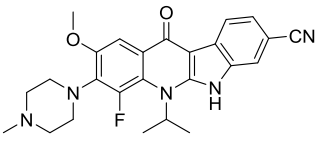
**9a**



**9b**



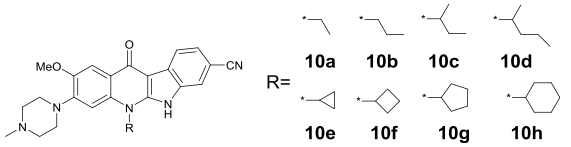
**8f**



**9c**

| compound | $IC_{50}$ (nM) (ALK inhibition) | $IC_{50}$ (nM) (cell growth inhibition in KARPAS-299) |
|---|---|---|
| **7d** | 21.3 ± 7.4 | 514.6 |
| **9a** | 19.8 ± 3.5 | 47.9 |
| **9b** | 6.6 ± 0.5 | 66.0 |
| **8f** | 4.8 ± 1.0 | 206.7 |
| **9c** | 8.9 ± 1.5 | 19.7 |

**Table 4. Effect of Different Substitutions on the Quinolone Nitrogen Atom**




| compound | $IC_{50}$ (nM) (ALK Inhibition) | $IC_{50}$ (nM) (cell growth inhibition in KARPAS-299) |
|---|---|---|
| **8f** | 4.8 ± 1.0 | 206.7 |
| **10a** | 20.6 ± 4.1 | >100,000 |
| **10b** | 13.5 ± 3.7 | 4957 |
| **10c** | 5.3 ± 2.1 | 271.8 |
| **10d** | 8.2 ± 2.1 | 147.5 |
| **10e** | 16.5 | 9884 |
| **10f** | 12.7 ± 1.7 | 138 |
| **10g** | 5.2 ± 0.7 | 129.8 |
| **10h** | 1.6 ± 0.3 | 151.6 |

inhibition of cell growth in the KARPAS-299 cell line. Compound **10g** has a similar ALK inhibitory activity as **8f**, whereas **10f** is 2 times less potent than **8f** in ALK inhibition, and **10h** is 3 times more potent than **8f**. However, these three compounds show very similar cell growth inhibition activity in the KARPAS-299 cell line when compared to **8f**.

Among those ALK inhibitors shown in Tables 1−4, compound **9c** potently inhibits ALK enzymatic activity with an $IC_{50}$ value of 8.9 nM and is also the most potent compound in inhibition of cell growth in the KARPAS-299 cell line with an $IC_{50}$ value of 19.7 nM. We performed further optimization of **9c** by focusing on modifications of its solubilizing group (Table 5).

We replaced the 1-methylpiperazine in **9c** (ALK-68) with 4-(piperidin-4-yl)morpholine, the same solubilizing group used in **3** (alectinib), yielding ALK-73. ALK-73 is similarly potent in inhibition of both ALK enzymatic activity and cell growth inhibition in the KARPAS-299 cell line, as compared to ALK-68.

We replaced 1-methylpiperazine in ALK-68 with *N*,*N*-dimethylpiperidin-4-amine, which yielded CJ-2355. CJ-2355 is potent in inhibition of ALK ($IC_{50}$ = 5.8 nM) but has a much weaker activity in inhibition of KARPAS-299 cell growth ($IC_{50}$ = 275 nM) than ALK-68. Although the reason why CJ-2355 was much less potent than ALK-68 in inhibition of cell growth needed further investigation, the data nevertheless indicated the critical importance of the solubilizing group at this site for achieving potent cellular activity. Accordingly, we have synthesized a series of compounds with diverse solubilizing groups.

Changing 1-methylpiperazine in ALK-68 with 1-methylpiperidine resulted in CJ-2212, which is more potent than ALK-68 in inhibition of ALK activity ($IC_{50}$ = 0.49 nM) and cell growth in the KARPAS-299 cell line ($IC_{50}$ = 9.7 nM).

Encouraged by the excellent potencies for CJ-2212, we replaced the 1-methyl group in CJ-2212 with oxetane, isopropyl, tetrahydro-2*H*-pyran, and ethan-1-ol, respectively, yielding CJ-CJ-2224, CJ-2225, CJ-2226, and CJ-2227, respectively. These four compounds all potently inhibit ALK activity with $IC_{50}$ values of 0.62−5.5 nM, with CJ-2224 being the least potent ($IC_{50}$ = 5.5 nM) among them. They also displayed potent cell growth inhibition in the KARPAS-299 cell line with $IC_{50}$ values of 17−96 nM, with CJ-2224 being the least potent ($IC_{50}$ = 96 nM).

We next synthesized a total of eight compounds containing a piperazine group substituted with one or more methyl group (CJ-2349, CJ-2350, CJ-2351, CJ-2356, CJ-2357, CJ-2358, CJ-2359, and CJ-2360). These eight compounds display potent and similar inhibitory activities of ALK with $IC_{50}$ values of 1.5−4.9 nM. While CJ-2349, CJ-2350, and CJ-2351 only display moderate cell growth inhibition activity in the KARPAS-299 cell line with $IC_{50}$ values of 116−162 nM, CJ-2356, CJ-2357, CJ-2358, CJ-2359, and CJ-2360 achieve potent cell growth inhibition with $IC_{50}$ values of 1.8−29 nM. Among them, CJ-2360 achieves an $IC_{50}$ value of 1.8 nM in inhibition of cell growth in the KARPAS-299 cell line. We further tested CJ-2360 for its potency in cell growth inhibition in the H3122 non-small-cell lung cell line carrying EML4-ALK and obtained an $IC_{50}$ value of 3 nM. CJ-2360 is thus the most potent ALK inhibitor among all


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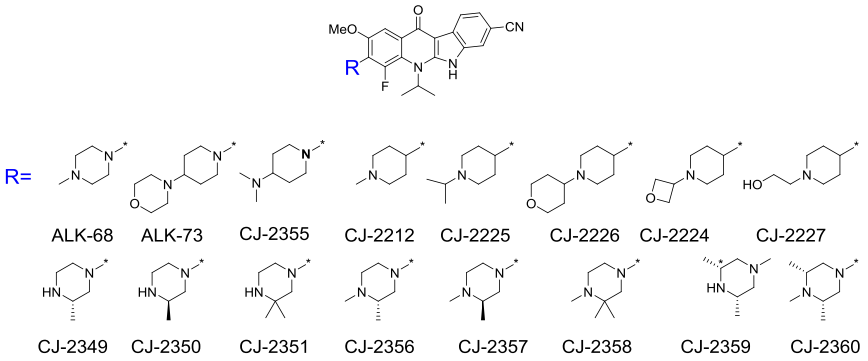
**Table 5. Investigation of the Structure−Activity Relationships of the Solubilizing Group**




| compound | $IC_{50}$ (nM) (ALK inhibition) | $IC_{50}$ (nM) (cell growth inhibition in KARPAS-299) | compound | $IC_{50}$ (nM) (ALK inhibition) | $IC_{50}$ (nM) (cell growth inhibition in KARPAS-299) |
|---|---|---|---|---|---|
| **9c** (ALK-68) | 8.9 ± 1.5 | 19.7 | CJ-2349 | 4.9 ± 1.4 | 162 |
| ALK-73 | 4.0 | 40.5 | CJ-2350 | 3.9 ± 1.6 | 116 |
| CJ-2355 | 5.8 | 275 | CJ-2351 | 2.6 ± 1.6 | 117 |
| CJ-2212 | 0.49 | 9.7 | CJ-2356 | 4.8 ± 0.8 | 29 |
| CJ-2225 | 2.5 | 17 | CJ-2357 | 2.2 ± 0.9 | 20 |
| CJ-2226 | 1.3 | 30 | CJ-2358 | 1.5 ± 0.2 | 5 |
| CJ-2224 | 5.5 | 96 | CJ-2359 | 2.6 ± 0.9 | 10 |
| CJ-2227 | 0.62 | 36 | CJ-2360 | 2.2 ± 0.3 | 1.8 |

the ALK inhibitors we have synthesized and evaluated in this study.

To understand the structural basis for its potent ALK inhibitory activity, we modeled the binding model for CJ-2360 in complex with ALK and compared the predicted binding model for CJ-2360 to the cocrystal structure of alectinib in complex with ALK (Figure 3). Overall, CJ-2360 has a very similar binding mode as compared to alectinib. However, there are some subtle differences for alectinib and CJ-2360 on their interactions with CJ-2360. For example, the ethyl group in alectinib has hydrophobic interactions with the side chains of Ala1200 and Leu1122, the corresponding methoxyl group in CJ-2360 maintains those hydrophobic interactions with the side chains of Ala1200 and Leu1122, and the oxygen atom in the methoxyl group has close contacts with the guanidinium group of Arg1120.

**Evaluation of Pharmacokinetics of CJ-2360.** Because CJ-2360 is the most potent ALK inhibitor we have identified based on its cell growth inhibitory activity in the KARPAS-299 cell line, we evaluated its pharmacokinetics of CJ-2360 in mice and rats, obtaining the results summarized in Table 6.

The PK data for CJ-2360 in mice showed that it achieves a reasonable oral exposure and an overall oral bioavailability of 38.2%. It has a modest clearance rate of 1.2 L/h/kg and a large volume distribution of 7.5 L/kg, suggesting extensive tissue distribution in mice.

CJ-2360 has a similar PK profile in rats as compared to that in mice. It achieves an overall oral bioavailability of 32.0%. It has a moderate clearance rate of 2.9 L/h/kg and a large volume distribution of 8.4 L/kg, suggesting extensive tissue distribution in rats.

Seeking an understanding on the ability of CJ-2360 to penetrate into tumor tissues, we performed an analysis of the drug concentrations in plasma and tumor tissue in mice bearing KARPAS-299 xenograft tumors (Table 7). Our data showed that CJ-2360 achieves good plasma exposure in mice following a single dose of oral administration. Significantly, the drug concentrations in the tumor tissue are >5 times higher than those in the plasma at different time points, indicating drug accumulation in the tumor.

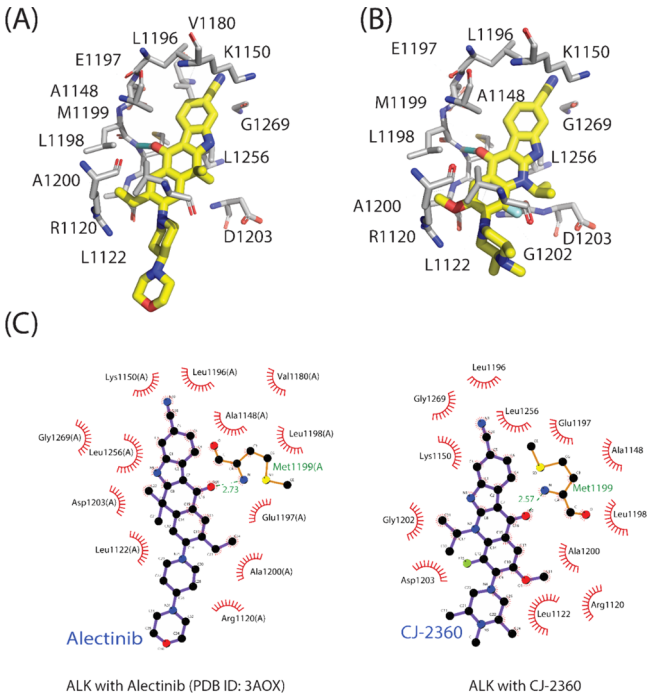



**Figure 3.** (A) Interaction residues in ALK with alectinib in previously reported cocrystal structure (PDB ID: 3AOX). (B) Interaction residues in ALK in our modeled structure of CJ2360 in complex with ALK. (C) Detailed hydrophobic and hydrogen bonding interactions between alectinib and ALK and between CJ-2360 and ALK, as analyzed by LigPlot.

We next tested CJ-2360 in KARPAS-299 xenograft tumors for its ability to inhibit ALK activity with alectinib included as a positive control in a pharmacodynamic (PD) experiment (Figure 4).


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**Table 6. Pharmacokinetic Parameters of CJ-2360 in Mice and Rats with Intravenous and Oral Administration**

| | dose (mg/kg) | | $C_{max}$ (ng/mL) | | $AUC_{0-t}$ (ng·h/mL) | | $t_{1/2}$ (h) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| CJ-2360 | IV | PO | IV | PO | IV | PO | IV | PO | CL (IV) (L/h/kg) | $V_{ss}$ (IV) (L/kg) | *F* (PO) |
| mouse | 2 | 20 | | 213 | 458 | 1783 | 2.1 | 7.5 | 1.2 | 7.5 | 38.2% |
| rat | 2 | 20 | | 202 | 639 | 2219 | 2.4 | 8.4 | 2.9 | 8.4 | 32.0% |

**Table 7. Analysis of CJ-2360 Concentrations in Mouse Plasma and Tumor Tissue[a]**

| PO (100 mg/kg) | concentration in plasma (ng/mL) | | | concentration in tumor (ng/g) | | | | |
|---|---|---|---|---|---|---|---|---|
| time point (h) | mouse 1 | mouse 2 | mean | right tumor | left tumor | right tumor | left tumor | mean |
| 1 | 2780 | 3540 | 3160 | 10,000 | 14,050 | 12,100 | 12,850 | 12,250 |
| 3 | 2220 | 7000 | 4610 | 25,600 | 23,000 | 32,600 | 37,000 | 29,550 |
| 6 | 890 | 850 | 870 | 28,000 | 22,600 | 38,200 | 38,400 | 31,800 |
| 24 | 9.26 | 24.3 | 16.78 | 990 | 895 | 1335 | 790 | 1003 |

[a]Mice bearing KARPAS-299 tumors were dosed with a single dose of CJ-2360 at 100 mg/kg via oral gavage and mice were sacrificed at indicated time points, with two mice for each time point. Each mouse had two tumors.

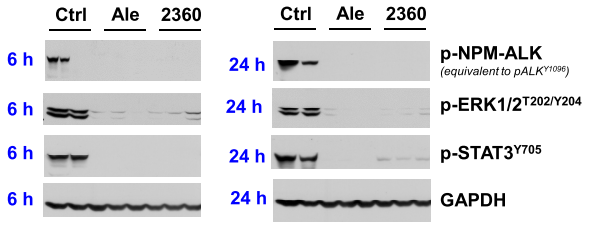


**Figure 4.** Pharmacodynamic study to evaluate CJ-2360 for its ability to inhibit ALK activity in KARPAS-299 tumors in mice. Mice were dosed with a single dose of CJ-2360 (2360) at 100 mg/kg or alectinib (Ale) at 20 mg/kg via oral gavage.

Our PD data showed that a single dose of CJ-2360 is very effective in inhibition of ALK phosphorylation, as well as ERK and STAT3 phosphorylation in KARPAS-299 tumor tissue, with the effect persisting for at least 24 h.

**Evaluation of CJ-2360 for Its Antitumor Efficacy *In Vivo*.** Based on its promising *in vivo* PD data and excellent tumor tissue exposure, we evaluated CJ-2360 for its antitumor efficacy in the KARPAS-299 xenograft model in mice. Since CJ-2360 effectively inhibited the AKT activity at the 6 h time point but had a slightly reduced inhibition of the AKT activity at the 24 h time point in our PD experiment (Figure 4), we chose twice daily dosing for testing its antitumor activity. Ceritinib was included as a positive control in this efficacy experiment because of its demonstrated excellent efficacy in the KARPAS-299 xenograft model.[26]

Our efficacy experiment showed that CJ-2360 is very efficacious in the KARPAS-299 xenograft model (Figure 5A). It achieves complete tumor regression in 100% of tumors (7 out of 7) and all tumors did not return until day 53, 23 days after the last dose. Hence, CJ-2360 is capable of achieving complete and long-lasting tumor regression in the KARPAS-299 xenograft tumor model. CJ-2360 is well tolerated in mice and did not cause weight loss (Figure 5B) or other signs of toxicity during the entire experiment. In our experiment, ceritinib is also very efficacious, but it only achieved complete tumor regression in 4 out of 7 tumors with 50 mg/kg, daily administration, suggesting that higher doses or a more frequent dosing schedule is needed for ceritinib to achieve 100% tumor regression in mice.

**Inhibitory Activity of CJ-2360 against Clinically Relevant ALK Mutants.** In clinic, patients with ALK-positive tumors treated with crizotinib (**1**) developed resistance to the drug due to generation of ALK mutations and a number of clinical ALK mutants have been identified.[27] We evaluated CJ-

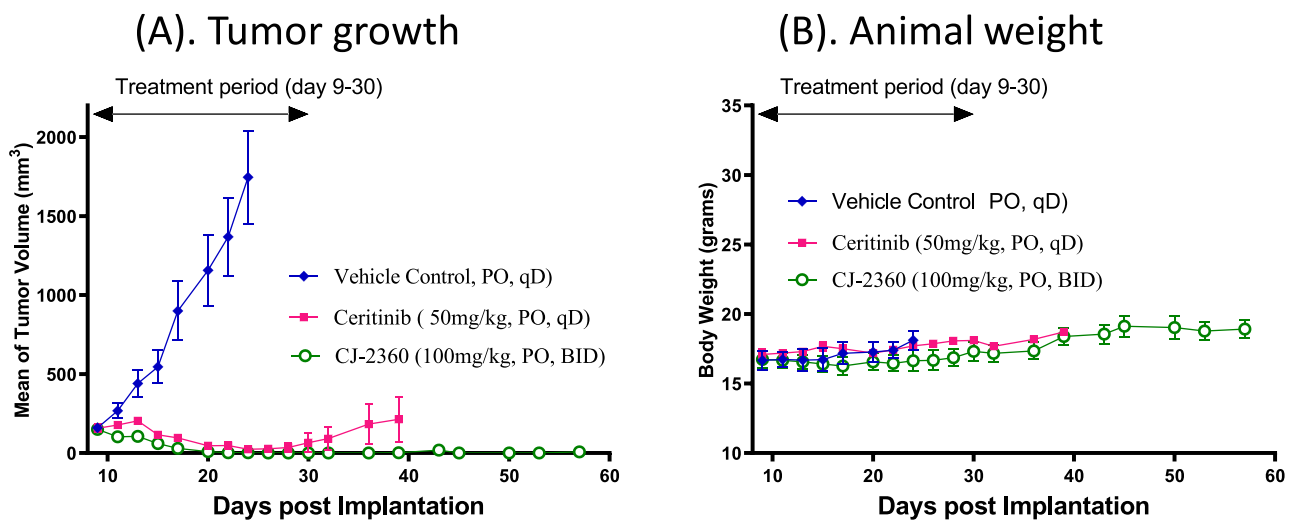


**Figure 5.** Antitumor activity of CJ-2360 in the KARPAS-299 xenograft tumor model. CJ-2360 was orally administered twice daily at 100 mg/kg and ceritinib was orally administered daily at 50 mg/kg for 22 days (from days 9 to 30).


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Table 8. Inhibitory Activity of Crizotinib, CJ-2360, Alectinib, and Ceritinib against Wild-Type ALK and Clinical ALK Mutants

| | $IC_{50}$ (nM) | | | | |
|---|---|---|---|---|---|
| compound | ALK wild type | ALK F1197M | ALK G1269A | ALK L1196M | ALK S1206Y |
| crizotinib | 34.5 ± 5.0 | 416 ± 43 | 1035 ± 194 | 1540 ± 270 | 1066 ± 232 |
| CJ-2360 | 2.2 ± 0.3 | 4.0 ± 1.4 | 8.8 ± 2.2 | 6.3 ± 2.2 | 8.9 ± 2.6 |
| alectinib | 0.59 ± 0.09 | 1.0 | 3.9 | 1.5 ± 0.3 | 0.96 ± 0.24 |
| ceritinib | 0.61 ± 0.02 | 3.5 | 1.9 | 1.1 ± 0.1 | 1.6 ± 0.3 |

2360, together with crizotinib (**1**), alectinib, and ceritinib, for its inhibitory activity against four clinically reported ALK mutants, namely, F1197M, G1269A, L1196M, and S1206Y, and obtained the results summarized in Table 8.

Our data showed that CJ-2360 demonstrates potent inhibitory activity with $IC_{50}$ values of 2.2, 4.0, 8.8, 6.3, and 8.9 nM against wild-type ALK and F1197M, G1269A, L1196M, and S1206Y ALK mutants, respectively. Crizotinib has $IC_{50}$ values of 34.5, 416, 1035, 1540, and 1066 nM against wild-type ALK and F1197M, G1269A, L1196M, and S1206Y ALK mutants, respectively. Alectinib has $IC_{50}$ values of 0.59, 1.0, 3.9, 1.5, and 0.96 nM against wild-type ALK and F1197M, G1269A, L1196M, and S1206Y ALK mutants, respectively, whereas ceritinib achieves $IC_{50}$ values of 0.61, 3.5, 1.9, 1.1, and 1.6 nM against wild-type ALK and F1197M, G1269A, L1196M, and S1206Y ALK mutants, respectively.

Hence, the fold of resistance for crizotinib ranges from 12 to 44 for these clinically relevant ALK mutants over wild-type ALK, whereas the fold of resistance for CJ-2360 is 2−4 for these clinically relevant ALK mutants over wild-type ALK. Alectinib and ceritinib have 2−6-fold resistance for these clinically relevant ALK mutants over wild-type ALK. CJ-2360 is thus >100 times more effective than crizotinib against these four clinically relevant ALK mutants.

**Selectivity of CJ-2360 against Other Human Kinases by KINOMEscan.** CJ-2360 was evaluated by KINOMEscan in DiscoverX for its inhibitory activity in a panel of 468 kinases (Table 9 and Table S1).

Table 9. Profiling the Selectivity of CJ-2360 against 468 Kinases by KINOMEscan

| kinase | $K_d$ (nM) | kinase | $K_d$ (nM) |
|---|---|---|---|
| ALK | 5.6 | FAK | >3000 |
| ALK (C1156Y) | 1.2 | IGF1R | >3000 |
| ALK (L1196M) | 4.8 | INSR | 950 |
| CLK1 | 31 | INSRR | 1600 |
| DAPK1 | 23 | LTK | 6.3 |
| DAPK2 | 22 | MERTK | 11 |
| DAPK3 | 260 | ROS1 | >3000 |

Consistent with the ALK inhibition data obtained in the ALK kinase assay developed in our laboratory, CJ-2360 achieves potent inhibitory activity against wild-type ALK with $IC_{50}$ = 5.6 nM. In addition, CJ-2360 is also potent against two clinically reported ALK mutants (C1156Y and L1196M) included in the 468 kinase panel.

Outside of ALK, CJ-2360 only inhibited 11 other non-ALK kinases at 100 nM with >50% inhibition (Table S1). CJ-2360 displays potent inhibitory activity against LTK (leukocyte receptor tyrosine kinase), which belongs to the same subfamily of the insulin receptor kinase superfamily (Table 9). CJ-2360 inhibits Mer tyrosine-protein kinase (MERTK), CLK1, DAPK1, DAPK2, and DAPK3 with $IC_{50}$ values of 6.3, 11, 31, 23, 22, and 260 nM, respectively (Table 9). CJ-2360 displays >100-fold selectivity for ALK over the insulin receptor kinase (INSR) and shows no significant activity against insulin-like growth factor 1 receptor (IGF1R), two kinases for which ALK belongs to the same subfamily of kinases. Hence, the KINOMEscan data showed that CJ-2360 is a potent inhibitor against wild-type ALK and clinically reported ALK mutants and displays potent inhibitory activity against only a few other kinases (LTK, MERTK, CLK1, DAPK1, and DAPK2) among the 468 kinases evaluated. Mer tyrosine kinase and DAPK kinases are considered as potential therapeutic targets for human cancer. Therefore, CJ-2360 may serve as a promising lead compound for these kinases.

Previously, alectinib was tested for its selectivity against 402 kinases at 10 and 1000 nM.[27] Alectinib inhibited 29 other non-ALK kinases with >95% inhibition at 1 $\mu$M among 402 kinases tested.[27] In our study, CJ-2360 only inhibited 11 other non-ALK kinases with >50% inhibition at 100 nM. Alectinib and CJ-2360 also inhibited the same kinases with different potencies. For example, alectinib inhibited GAK with 79% inhibition at 10 nM ($IC_{50}$ < 10 nM) and CJ-2360 inhibited GAK with 59% inhibition at 100 nM. Taken together, our data showed that CJ-2360 is a highly selective ALK inhibitor.

## CHEMISTRY

The synthesis of compounds **7a−7d** is outlined in Scheme 1. The intermediate methyl 2-(phenylamino)-1*H*-indole-3-carboxylates (**13a−13c**) were synthesized according to published methods[33] with minor modifications. Compounds **13a−13c** are obtained by chlorination of **12b** with NCS in the presence of DABCO, followed by addition of the aniline as its trichloroacetate.

Cyclization of **13a−13c** in boiling diphenyl ether affords the corresponding 5,6-dihydro-11*H*-indolo[2,3-*b*]quinolin-11-one (**14a−14c**).[33] Substitution with 4-morpholinopiperidine or 1-methylpiperazine in DMSO in the presence of DIPEA affords compounds **7a−7d**.

The synthesis of compounds **8a−8e** is outlined in Scheme 2. Methyl 2-((4-bromo-3-fluorophenyl)(isopropyl)amino)-6-cyano-1*H*-indole-3-carboxylate (**15**) is obtained by chlorination of **12b** with NCS in the presence of DABCO, followed by addition of the aniline as the trichloroacetate. Cyclization of **15** in boiling diphenyl ether affords 5,6-dihydro-11*H*-indolo[2,3-*b*]quinolin-11-one (**16**). Reaction with 1-methylpiperazine in DMSO in the presence of DIPEA afforded the designed compound (**8a**). Compounds **8b−8e** were obtained by Suzuki coupling of **8a** with appropriate 4,4,5,5-tetramethyl-1,3,2-dioxaborolanes, followed by hydrogenation in THF.

The synthesis of compounds **8f−8i** is outlined in Scheme 3. Alkoxyl-substituted methyl 2-((4-alkoxyl-3-fluorophenyl)-(isopropyl)amino)-6-cyano-1*H*-indole-3-carboxylates (**17a−17d**) are obtained by chlorination of **12b** with NCS in the presence of DABCO, followed by addition of the aniline as its trichloroacetate. Cyclization of **17a−17d** in boiling diphenyl


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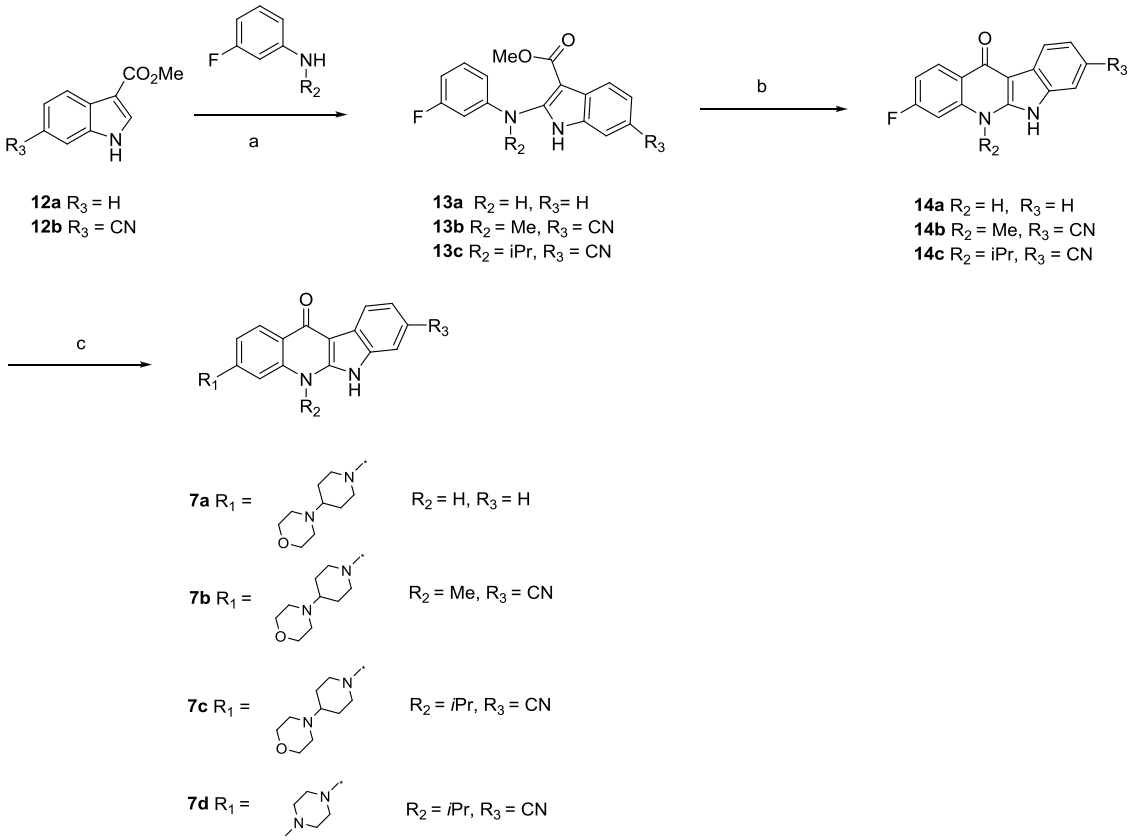
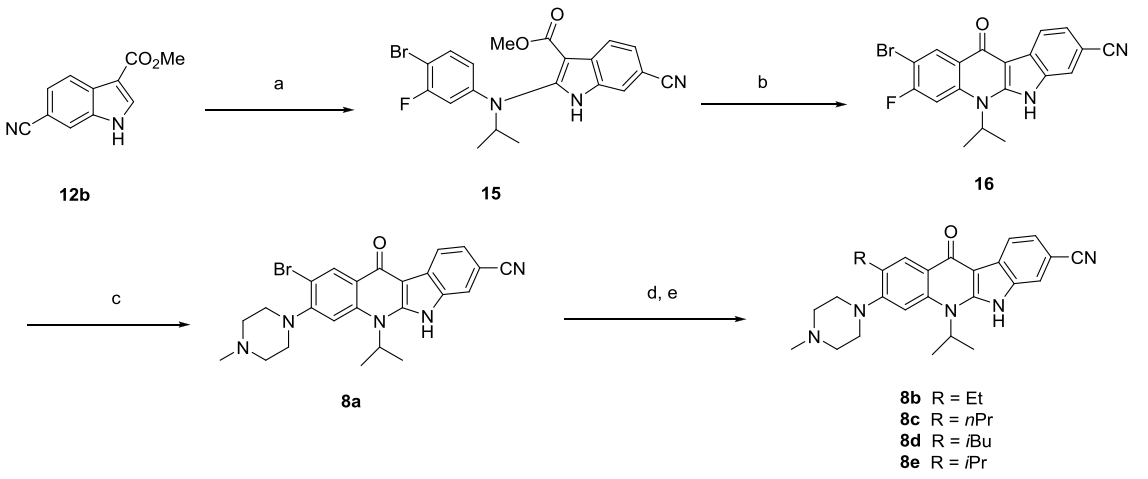
**Scheme 1. Synthesis of 7a−7d**[a]




[a]Reagents and conditions: (a) (i) NCS, DABCO, DCM, 0 °C, 2 h; (ii) trichloroacetic acid, DCM, RT, 2 h; (b) $Ph_2O$, reflux, 1 h; (c) 4-morpholinopiperidine or 1-methylpiperazine, DIPEA, DMSO, 120−140 °C, 3 days.

**Scheme 2. Synthesis of 8a−8e**[a]




[a]Reagents and conditions: (a) (i) NCS, DABCO, DCM, 0 °C, 2 h; (ii) 4-bromo-3-fluoro-*N*-isopropylaniline, trichloroacetic acid, DCM, RT, 2 h; (b) $Ph_2O$, reflux, 1 h; (c) 1-methylpiperazine, DIPEA, DMSO, 120−140 °C, 3 days; (d) (i) appropriate 4,4,5,5-tetramethyl-1,3,2-dioxaborolane, $Pd(PPh_3)_2Cl_2$, $Na_2CO_3$, DME/$H_2O$, 80 °C; (ii) 10% Pd-C, $H_2$, THF.

ether affords the alkoxyl-substituted 5,6-dihydro-11*H*-indolo-[2,3-*b*]quinolin-11-ones (**18a−18d**). Substitution with 1-methylpiperazine in DMSO in the presence of DIPEA affords the designed compounds (**8f−8i**).

The synthesis of compounds **9a−9c** is outlined in Scheme 4. Di- or trifluoro-substituted methyl 6-cyano-2-(isopropyl-(phenyl)amino)-1*H*-indole-3-carboxylates (**19a−19c**) are obtained by chlorination of **12b** with NCS in the presence of DABCO, followed by addition of the appropriate aniline as its trichloroacetate. Cyclization of **19a−19c** in boiling diphenyl ether affords the di- or trifluoro-substituted 5,6-dihydro-11*H*-indolo[2,3-*b*]quinolin-11-ones (**20a−20c**), and substitution with 1-methylpiperazine in DMSO in the presence of DIPEA affords the designed compounds (**9a−9c**). The synthesis of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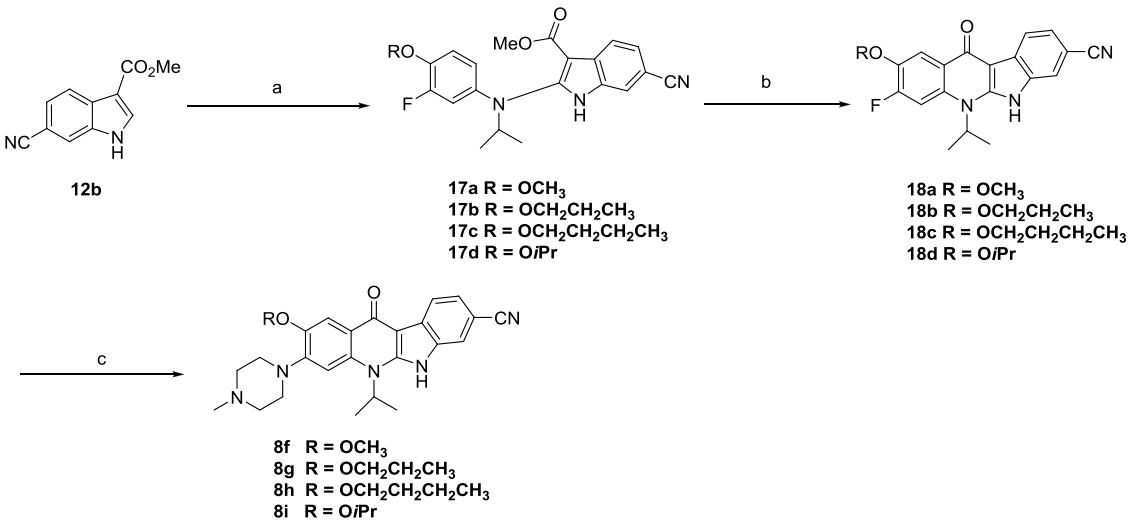
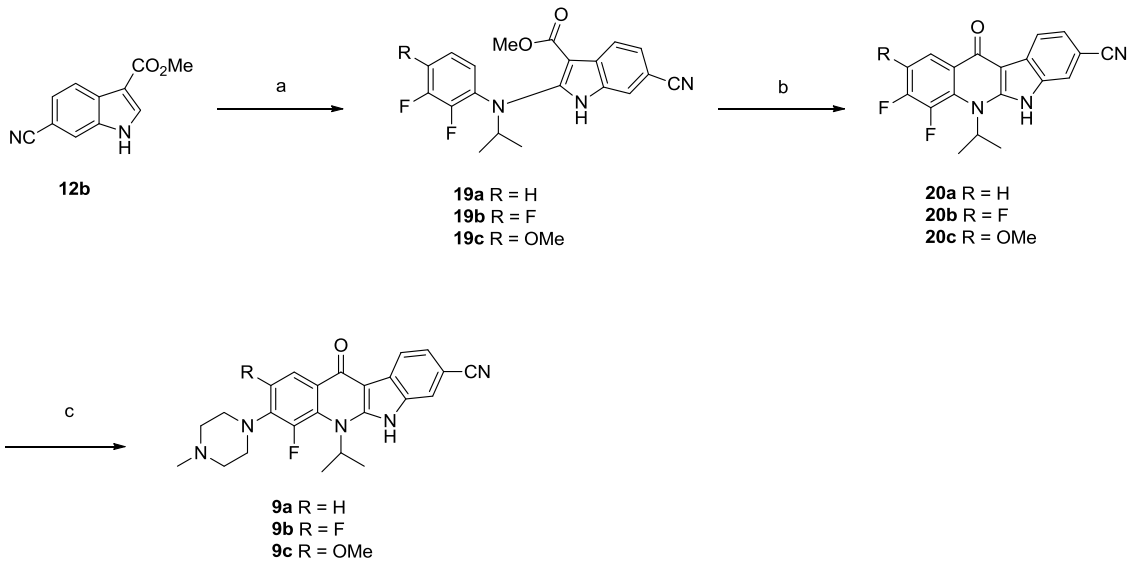
**Scheme 3. Synthesis of 8f−8i**[a]




[a]Reagents and conditions: (a) (i) NCS, DABCO, SCM, 0 °C, 2 h; (ii) trichloroacetic acid; appropriate anilines, RT, 2 h; (b) $Ph_2O$, reflux, 1 h; (c) 1-methylpiperazine, DIPEA, DMSO, 120−140 °C, 3 days.

**Scheme 4. Synthesis of 9a−9c**[a]




[a]Reagents and conditions: (a) (i) NCS, DABCO, DCM, 0 °C, 2 h; (ii) trichloroacetic acid, appropriate anilines, RT, 2 h; (b) $Ph_2O$, reflux, 1 h; (c) 1-methylpiperazine, DIPEA, DMSO, 120−140 °C, 3 days.

compounds **10a−10h** is outlined in Scheme 5. Methyl 6-cyano-2-(phenylamino)-1*H*-indole-3-carboxylates (**23a−23h**) are obtained by chlorination of methyl 6-cyano-1*H*-indole-3-carboxylate with NCS in the presence of DABCO, followed by addition of the appropriate alkyl- or cycloalkyl-substituted aniline as the trichloroacetate. Cyclization of **23a−23h** in boiling diphenyl ether affords alkyl- or cycloalkyl-substituted 5,6-dihydro-11*H*-indolo[2,3-*b*]quinolin-11-ones (**24a−24h**). Substitution with 1-methylpiperazine in DMSO in the presence of DIPEA affords the designed compounds (**10a−10h**).

The synthesis of compounds ALK-73, CJ-2349-2351, and CJ-2356-2360 is outlined in Scheme 6. Compound **20c** was substituted with 1-methylpiperazine in DMSO in the presence of DIPEA at 120−140 °C for 3 days to give the designed compounds.

The synthesis of compounds CJ-2212 and CJ-2224-2227 is outlined in Scheme 7. Nucleophilic substitution of F in **25** by sodium methoxide gave anisole **26**. Aniline **28** was obtained by Suzuki coupling of **26** followed by hydrogenation. Reductive amination of **28** afforded isopropyl-substituted aniline **29**. Methyl 6-cyano-2-(phenylamino)-1*H*-indole-3-carboxylate **30** was obtained by chlorination of methyl 6-cyano-1*H*-indole-3-carboxylate with NCS in the presence of DABCO, followed by addition of substituted aniline **29** and trichloroacetate. Deprotection of Boc in **30** followed by reductive amination gave piperidine **31b−31f**. Cyclization of **31b−31f** in boiling diphenyl ether affords target molecules.

## CONCLUSIONS

We have designed, synthesized, and evaluated a new class of ALK inhibitors containing a fused indoloquinoline scaffold. Our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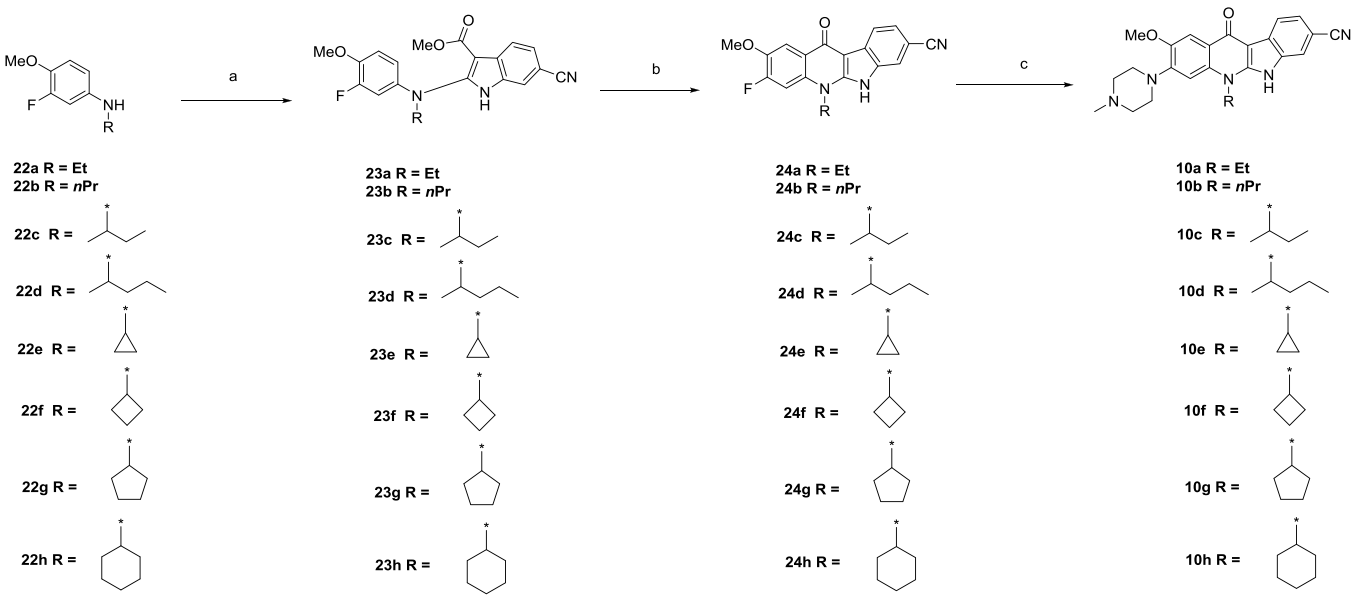
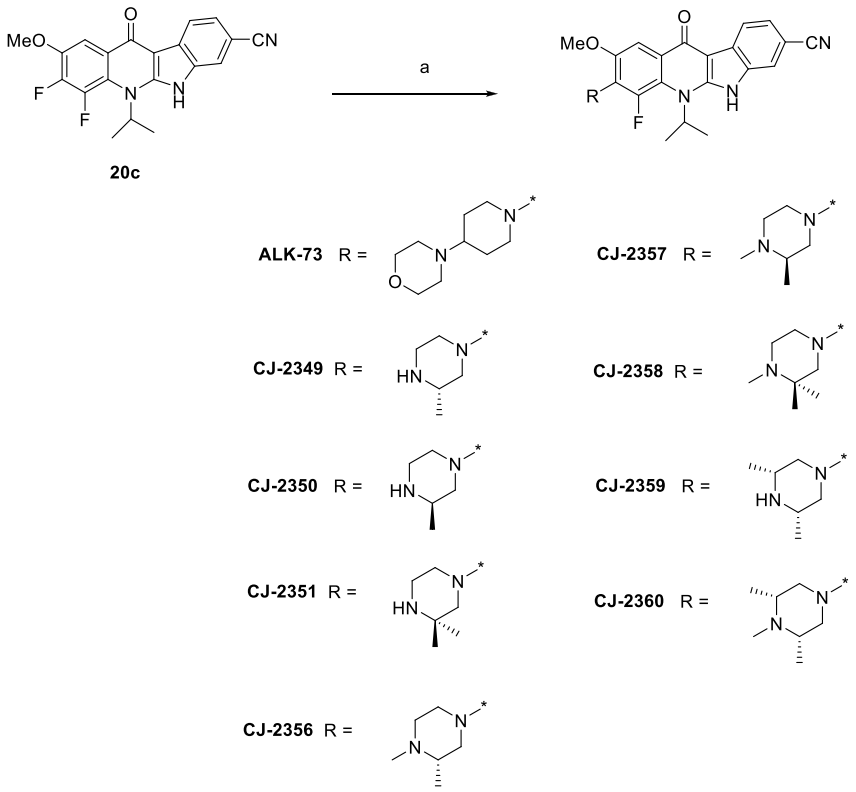
**Scheme 5. Synthesis of 10a−10h**[a]




[a]Reagents and conditions: (a) (i) methyl 6-cyano-1*H*-indole-3-carboxylate, NCS, DABCO, DCM, 0 °C, 2 h; (ii) trichloroacetic acid, appropriate anilines, RT, 2 h; (b) $Ph_2O$, reflux, 1 h; (c) 1-methylpiperazine, DIPEA, DMSO, 120−140 °C, 3 days.

**Scheme 6. Synthesis of ALK-73, CJ-2349-2351, and CJ-2356-2360**[a]




[a]Reagents and conditions: (a) appropriate amines, DIPEA, DMSO, 120−140 °C, 3 days.

efforts have resulted in the discovery of CJ-2360 as a potent, orally bioavailable, and highly efficacious ALK inhibitor. CJ-2360 effectively inhibits wild-type ALK activity with an $IC_{50}$ = 2.2 nM. Importantly, CJ-2360 displays potent inhibitory activity against four clinically reported ALK mutants with $IC_{50}$ values of 4.4−8.9 nM and is >100 times more potent than crizotinib against these clinical ALK mutants. CJ-2360 potently inhibits cell growth in the ALK-positive KARPAS-299 anaplastic large-cell lymphoma cell line carrying NPM-ALK fusion and the H3122 non-small-cell lung cell line carrying EML4-ALK with $IC_{50}$ values of 2 and 3 nM, respectively. CJ-2360 has good oral pharmacokinetics in mice and rats. CJ-2360 effectively inhibits ALK activity in the ALK-positive KARPAS-299 xenograft tumor with a single administration in mice. CJ-2360 is capable of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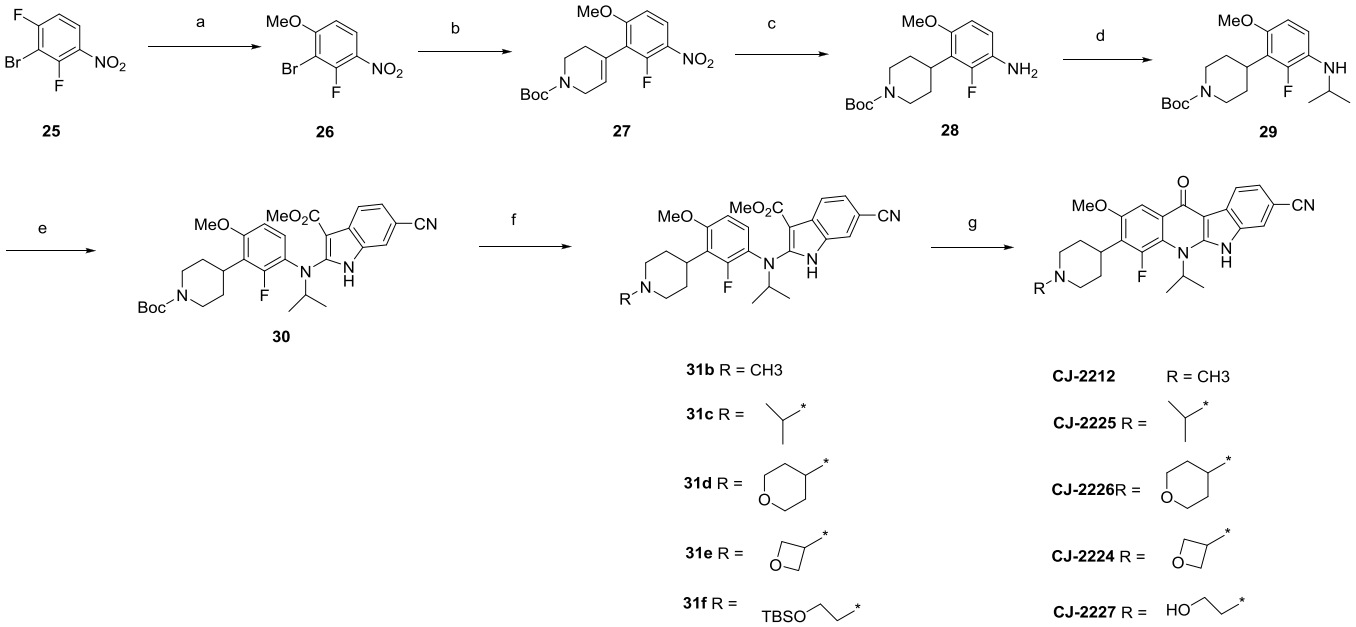
**Scheme 7. Synthesis of CJ-2212 and CJ-2224-2227**[a]




[a]Reagents and conditions: (a) MeONa, MeOH, 0 °C to RT, 4 h, 55%; (b) *tert*-butyl 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-5,6-dihydropyridine-1(2*H*)-carboxylate, $Pd(dppf)Cl_2$, and $K_2CO_3$, 92%; (c) 10% Pd-C, ethanol, RT, overnight, 92%; (d) acetone, acetic acid, $NaBH(OAc)_3$, DCM, RT, 12 h, 94%; (e) (i) methyl 6-cyano-1*H*-indole-3-carboxylate, NCS, DABCO, DCM, 0 °C, 2 h; (ii) trichloroacetic acid, RT, 2 h, 69%; (f) (i) TFA; (ii) appropriate aldehyde or ketone, $NaBH(OAc)_3$, acetic acid, DCM; (g) $Ph_2O$, reflux, 1 h.

achieving complete and long-lasting tumor regression in the KARPAS-299 xenograft model in mice at a well-tolerated dose schedule. Collectively, our data demonstrate that CJ-2360 is a promising ALK inhibitor for advanced preclinical development.

## EXPERIMENTAL SECTION

**General Methods.** Solvents and reagents were obtained commercially and used without further purification. Reactions were monitored by TLC on 250 $\mu$m E. Merck silica gel plates (60F-254) using UV light for visualization. E. Merck silica gel (60; particle size, 15−40 $\mu$m) was used for flash column chromatography. The NMR spectra were recorded on a Bruker Avance300 spectrometer (300 MHz) and Bruker Avance III spectrometer (400 MHz). Chemical shifts are reported as $\delta$ values (ppm) downfield relative to TMS as an internal standard, with multiplicities reported in the standard form. All final compounds have purities >95%, as determined by HPLC (UV detection at 254 nm).

**Methyl 2-((3-Fluorophenyl)amino)-1*H*-indole-3-carboxylate (13a).** DABCO (62 mg, 0.55 mmol) was added to a solution of methyl 1*H*-indole-3-carboxylate (175 mg, 1 mmol) in DCM (20 mL) and the mixture was cooled down to 0 °C. NCS (147 mg, 1.1 mmol) was then added and the reaction was stirred at 0 °C for 2 h. A solution of 3-fluoroaniline (111 mg, 1 mmol) and trichloroacetic acid (41 mg, 0.25 mmol) in DCM (5 mL) was added dropwise and the reaction was stirred for 2 h at RT. The reaction was quenched by adding $H_2O$ and the mixture was extracted with DCM. Solvent was removed under vacuum and the residue was purified by silica gel chromatography with hexane/EtOAc (4/1, v/v) to afford the title compound as a colorless oil (200 mg, 70% yield). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ ppm 9.14 (s, 1H), 8.30 (s, 1H), 7.88−7.81 (m, 1H), 7.43−7.37 (m, 1H), 7.30−7.16 (m, 2H), 7.16−6.95 (m, 3H), 6.94−6.87 (m, 1H), 3.98 (s, 3H).

**Methyl 6-Cyano-2-((3-fluorophenyl)(methyl)amino)-1*H*-indole-3-carboxylate (13b).** DABCO (62 mg, 0.55 mmol) was added to a solution of methyl 6-cyano-1*H*-indole-3-carboxylate (200 mg, 1 mmol) in DCM (20 mL) and the mixture was cooled down to 0 °C. NCS (147 mg, 1.1 mmol) was then added and the reaction was stirred at 0 °C for 2 h. A solution of 3-fluoro-*N*-methylaniline (125 mg, 1 mmol) and trichloroacetic acid (41 mg, 0.25 mmol) in DCM (5 mL) was added dropwise and the reaction was stirred for 2 h at RT. The reaction was quenched by adding $H_2O$ and the mixture was extracted with DCM. Solvent was removed under vacuum and the residue was purified by silica gel chromatography with hexane/EtOAc (4/1, v/v) to afford the title compound as a colorless oil (220 mg, 68% yield). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ ppm 8.26 (s, 1H), 8.03 (d, $J$ = 8.6 Hz, 1H), 7.46 (d, $J$ = 1.8 Hz, 1H), 7.39 (dd, $J$ = 8.6, 1.8 Hz, 1H), 7.21−7.18 (m, 1H), 6.66−6.49 (m, 3H), 3.85 (s, 3H), 3.43 (s, 3H).

**Methyl 6-Cyano-2-((3-fluorophenyl)(isopropyl)amino)-1*H*-indole-3-carboxylate (13c).** DABCO (62 mg, 0.55 mmol) was added to a solution of methyl 6-cyano-1*H*-indole-3-carboxylate (200 mg, 1 mmol) in DCM (20 mL) and the mixture was cooled down to 0 °C. NCS (147 mg, 1.1 mmol) was then added and the reaction was stirred at 0 °C for 2 h. A solution of 3-fluoro-*N*-isopropylaniline (153 mg, 1 mmol) and trichloroacetic acid (41 mg, 0.25 mmol) in DCM (5 mL) was added dropwise and the reaction was stirred for 2 h at RT. The reaction was quenched by adding $H_2O$ and the mixture was extracted with DCM. Solvent was removed under vacuum and the residue was purified by silica gel chromatography with hexane/EtOAc (4/1, v/v) to afford the title compound as a colorless oil (265 mg, 75% yield). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ ppm 8.39 (s, 1H), 8.23 (d, $J$ = 8.4 Hz, 1H), 7.64−7.59 (m, 1H), 7.50 (dd, $J$ = 8.4, 1.5 Hz, 1H), 7.24−7.22 (m, 1H), 6.74−6.64 (m, 1H), 6.60−6.56 (m, 2H), 4.56−4.49 (m, 1H), 3.81 (s, 3H), 1.32 (d, $J$ = 6.6 Hz, 6H).

**3-Fluoro-5,6-dihydro-11*H*-indolo[2,3-*b*]quinolin-11-one (14a).** Methyl 2-((3-fluoro-phenyl)amino)-1*H*-indole-3-carboxylate (193 mg, 0.68 mmol) was dissolved in diphenyl ether (10 mL) and the solution was refluxed for 1 h. The mixture was then cooled down to RT. Hexane (20 mL) was added and the precipitate was collected by filtration to afford the title compound as a pale yellow solid (82 mg, 48% yield). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 12.41 (s, 1H), 11.80 (s, 1H), 8.32 (dd, $J$ = 8.9, 6.6 Hz, 1H), 8.16 (d, $J$ = 6.6 Hz, 1H), 7.48 (d, $J$ = 7.7 Hz, 1H), 7.40 (dd, $J$ = 10.5, 2.5 Hz, 1H), 7.30−7.10 (m, 3H).

**3-Fluoro-5-methyl-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]-quinoline-8-carbonitrile (14b).** Methyl 6-cyano-2-((3-fluorophenyl)(methyl)amino)-1*H*-indole-3-carboxylate (220 mg, 0.68 mmol) was dissolved in diphenyl ether (10 mL) and the solution was refluxed for 1 h. The mixture was cooled down to RT. Hexane (20 mL) was added and the precipitate was collected by filtration to afford the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title compound as a pale yellow solid (178 mg, 90% yield). This product was used directly in the next step without further purification. MS: $m/z$ = 292 [M + H].

**3-Fluoro-5-isopropyl-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (14c).** Methyl 6-cyano-2-((3-fluorophenyl)(isopropyl)amino)-1*H*-indole-3-carboxylate (265 mg, 0.75 mmol) was dissolved in diphenyl ether (10 mL) and the solution was refluxed for 1 h. The mixture was cooled down to RT. Hexane (20 mL) was added and the precipitate was collected by filtration. The precipitate was purified by silica gel chromatography with hexane/EtOAc (2/1, v/v) to afford the title compound as a pale yellow solid (190 mg, 79% yield). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ ppm 12.24 (s, 1H), 8.45 (dd, $J$ = 8.8, 7.1 Hz, 1H), 8.32 (dd, $J$ = 7.9, 2.7 Hz, 1H), 8.05−7.84 (m, 2H), 7.66−7.64 (m, 1H), 7.34−7.25 (m, 1H), 5.35−5.32 (m, 1H), 1.75 (d, $J$ = 7.1 Hz, 6H).

**3-(4-Morpholinopiperidin-1-yl)-5,6-dihydro-11*H*-indolo-[2,3-*b*]quinolin-11-one (7a).** 4-Morpholinopiperidine (262 mg, 1.56 mmol) and DIPEA (0.5 mL) were added to a solution of 3-fluoro-5*H*-indolo[2,3-*b*]quinolin-11(6*H*)-one (130 mg, 0.52 mmol) in DMSO (2 mL) and the mixture was heated to 120−140 °C for 3 days. The reaction mixture was cooled down to RT and purified by preparative HPLC to afford the title compound as a pale yellow solid (60 mg, 29% yield). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 12.40 (s, 1H), 11.84 (s, 1H), 10.02 (s, 1H), 8.18−8.11 (m, 2H), 7.46 (d, $J$ = 7.7 Hz, 1H), 7.28−7.14 (m, 2H), 7.09 (dd, $J$ = 9.1, 2.3 Hz, 1H), 7.02 (d, $J$ = 2.3 Hz, 1H), 4.00−3.85 (m, 8H), 3.75−3.68 (m, 2H), 3.55−3.40 (m, 3H), 3.18−3.08 (m, 2H), 2.91 (t, $J$ = 12.5 Hz, 2H), 2.23−2.14 (m, 2H), 1.78−1.64 (m, 2H).

**5-Methyl-3-(4-morpholinopiperidin-1-yl)-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (7b).** 4-Morpholinopiperidine (262 mg, 1.56 mmol) and DIPEA (0.5 mL) were added to a solution of 3-fluoro-5-methyl-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (150 mg, 0.52 mmol) in DMSO (2 mL) and the mixture was heated to 120−140 °C for 3 days. The reaction mixture was cooled down to RT and purified by preparative HPLC to afford the title compound as a pale yellow solid (70 mg, 31% yield). $^1$H NMR (300 MHz, $CD_3OD$) $\delta$ ppm 8.30 (d, $J$ = 7.0 Hz, 1H), 8.28 (d, $J$ = 8.0 Hz, 1H), 7.58 (s, 1H), 7.52 (d, $J$ = 8.0 Hz, 1H), 7.12 (d, $J$ = 7.0 Hz,1H), 6.84 (s, 1H), 4.37−4.10 (m, 4H), 4.00−3.72 (m, 2H), 3.89 (s, 3H), 3.70−3.20 (m, 5H), 3.18−3.00 (m, 2H), 2.40−2.26 (m, 2H), 1.97−1.86 (m, 2H).

**5-Isopropyl-3-(4-morpholinopiperidin-1-yl)-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (7c).** 4-Morpholinopiperidine (262 mg, 1.56 mmol) and DIPEA (0.5 mL) were added to a solution of 3-fluoro-5-isopropyl-11-oxo-6,11-dihydro-5*H*-indolo-[2,3-*b*]quinoline-8-carbonitrile (190 mg, 0.59 mmol) in DMSO (2 mL) and the mixture was heated to 120−140 °C for 3 days. The reaction mixture was cooled down to RT and purified by preparative HPLC to afford the title compound as a pale yellow solid (75 mg, 27% yield). $^1$H NMR (300 MHz, $CD_3OD$) $\delta$ ppm 8.39 (d, $J$ = 8.0 Hz, 1H), 8.38 (d, $J$ = 8.9 Hz, 1H), 7.82 (s, 1H), 7.56 (dd, $J$ = 8.0, 1.3 Hz, 1H), 7.30−7.15 (m, 2H), 5.45−5.32 (m, 1H), 4.38−4.10 (m, 4H), 3.90−3.72 (m, 2H), 3.70−3.20 (m, 5H), 3.18−3.00 (m, 2H), 2.40−2.30 (m, 2H), 1.97−1.80 (m, 2H), 1.87 (d, $J$ = 7.1 Hz, 6H).

**5-Isopropyl-3-(4-methylpiperazin-1-yl)-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]Quinoline-8-Carbonitrile (7d).** 1-Methylpiperazine (0.5 mL) and DIPEA (0.5 mL) were added to a solution of 3-fluoro-5-isopropyl-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (190 mg, 0.59 mmol) in DMSO (2 mL) and the mixture was heated to 120−140 °C for 3 days. The reaction mixture was cooled down to RT and purified by preparative HPLC to afford the title compound as a pale yellow solid (48 mg, 20% yield). $^1$H NMR (300 MHz, $CD_3OD$) $\delta$ ppm 8.42 (d, $J$ = 8.1 Hz, 1H), 8.39 (d, $J$ = 9.2 Hz, 1H), 7.81 (d, $J$ = 1.3 Hz, 1H), 7.55 (dd, $J$ = 8.1, 1.3 Hz, 1H), 7.28 (d, $J$ = 1.8 Hz, 1H), 7.21 (dd, $J$ = 9.2, 1.8 Hz, 1H), 5.50−5.30 (m, 1H), 4.36−4.10 (m, 2H), 3.80−3.60 (m, 2H), 3.55−3.20 (m, 4H), 3.04 (s, 3H), 1.87 (d, $J$ = 7.1 Hz, 6H).

**Methyl 2-((4-Bromo-3-fluorophenyl)(isopropyl)amino)-6-cyano-1*H*-indole-3-carboxylate (15).** DABCO (62 mg, 0.55 mmol) was added to a solution of methyl 6-cyano-1*H*-indole-3-carboxylate (200 mg, 1 mmol) in DCM (20 mL) and the mixture was cooled down to 0 °C. NCS (147 mg, 1.1 mmol) was then added and the reaction was stirred at 0 °C for 2 h. A solution of 4-bromo-3-fluoro-*N*-isopropylaniline (232 mg, 1 mmol) and trichloroacetic acid (41 mg, 0.25 mmol) in DCM (5 mL) was added dropwise and the reaction was stirred for 2 h at RT. The reaction was quenched by adding $H_2O$ and the mixture was extracted with DCM. Solvent was removed under vacuum and the residue was purified by silica gel chromatography with hexane/EtOAc (4/1, v/v) to afford the title compound as a colorless oil (245 mg, 57% yield). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ ppm 8.44 (s, 1H), 8.27 (d, $J$ = 8.3 Hz, 1H), 7.73−7.64 (m, 1H), 7.54 (dd, $J$ = 8.3, 1.4 Hz, 1H), 7.34 (dd, $J$ = 9.0, 7.9 Hz, 1H), 6.56 (dd, $J$ = 11.3, 2.7 Hz, 1H), 6.41 (dd, $J$ = 9.0, 2.7 Hz, 1H), 4.45−4.41 (m, 1H), 3.81 (s, 3H), 1.31 (d, $J$ = 6.6 Hz, 6H).

**2-Bromo-3-fluoro-5-isopropyl-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (16).** Methyl 2-((4-bromo-3-fluorophenyl)(isopropyl)amino)-6-cyano-1*H*-indole-3-carboxylate (245 mg, 0.57 mmol) was dissolved in diphenyl ether (10 mL) and the solution was refluxed for 1 h. The mixture was cooled down to RT. Hexane (20 mL) was added and the precipitate was collected by filtration. The precipitate was purified by silica gel chromatography with hexane/EtOAc (2/1, v/v) to afford the title compound as a pale yellow solid (110 mg, 49% yield). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 12.30 (s, 1H), 8.54 (d, $J$ = 8.3 Hz, 1H), 8.28 (d, $J$ = 8.0 Hz, 1H), 8.08 (d, $J$ = 11.9 Hz, 1H), 7.92 (d, $J$ = 1.4 Hz, 1H), 7.63 (dd, $J$ = 8.0, 1.4 Hz, 1H), 5.37−5.28 (m, 1H), 1.74 (d, $J$ = 7.0 Hz, 6H).

**2-Bromo-5-isopropyl-3-(4-methylpiperazin-1-yl)-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (8a).** 1-Methylpiperazine (0.5 mL) and DIPEA (0.5 mL) were added to a solution of 2-bromo-3-fluoro-5-isopropyl-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (110 mg, 0.28 mmol) in DMSO (2 mL) and the mixture was heated to 120−140 °C for 3 days. The reaction mixture was cooled down to RT and purified by preparative HPLC to afford the title compound as a pale yellow solid (29 mg, 22% yield). $^1$H NMR (300 MHz, $CD_3OD$) $\delta$ ppm 8.58 (s, 1H), 8.29 (d, $J$ = 7.9 Hz, 1H), 7.77 (s, 1H), 7.52 (d, $J$ = 7.9 Hz, 1H), 7.49 (s, 1H), 5.44−5.30 (m, 1H), 3.90−3.70 (m, 4H), 3.60−3.30 (m, 4H), 3.08 (s, 3H), 1.89 (d, $J$ = 7.1 Hz, 6H).

**2-Ethyl-5-isopropyl-3-(4-methylpiperazin-1-yl)-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (8b).** A mixture of 2-bromo-5-isopropyl-3-(4-methylpiperazin-1-yl)-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (30 mg, 0.063 mmol), $Na_2CO_3$ (25 mg, 0.183 mmol), $Pd(PPh_3)_2Cl_2$ (4.3 mg, 0.0061 mmol), and 4,4,5,5-tetramethyl-2-vinyl-1,3,2-dioxaborolane (14 mg, 0.091 mmol) in DME/$H_2O$ (4.8 mL, 5/1, v/v) was stirred under nitrogen at 80 °C overnight. After the reaction was complete as indicated by LC−MS, the mixture was purified by preparative HPLC to afford 5-isopropyl-3-(4-methylpiperazin-1-yl)-11-oxo-2-vinyl-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile, which was dissolved in THF (5 mL). Pd/C (10%, 20 mg) was added to the THF solution and the mixture underwent hydrogenation at RT overnight until LC−MS indicated that the reaction was complete. The filtrate after filtration was concentrated and the residue was purified by preparative HPLC to afford the title compound as a pale yellow solid (14 mg, 52% yield for two steps). $^1$H NMR (300 MHz, $CD_3OD$) $\delta$ ppm 8.38 (s, 1H), 8.29 (d, $J$ = 8.0 Hz, 1H), 7.70 (s, 1H), 7.50 (s, 1H), 7.44 (d, $J$ = 8.0 Hz, 1H), 5.40−5.30 (m, 1H), 3.75−3.65 (m, 2H), 3.63−3.25 (m, 6H), 3.06 (s, 3H), 2.90 (q, $J$ = 7.4 Hz, 2H), 1.88 (d, $J$ = 7.1 Hz, 6H), 1.40 (t, $J$ = 7.4 Hz, 3H).

**5-Isopropyl-3-(4-methylpiperazin-1-yl)-11-oxo-2-propyl-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (8c).** A mixture of 2-bromo-5-isopropyl-3-(4-methylpiperazin-1-yl)-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (30 mg, 0.061 mmol), $Na_2CO_3$ (25 mg, 0.183 mmol), $Pd(PPh_3)_2Cl_2$ (4.3 mg, 0.0061 mmol), and 4,4,5,5-tetramethyl-2-(prop-1-en-1-yl)-1,3,2-dioxaborolane (16 mg, 0.091 mmol) in DME/$H_2O$ (4.8 mL, 5/1, v/v) was stirred under $N_2$ at 80 °C overnight. After the reaction was complete as indicated by LC−MS, the mixture was purified by preparative HPLC to afford 5-isopropyl-3-(4-methylpiperazin-1-yl)-11-oxo-2-(prop-1-en-1-yl)-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile, which was dissolved in THF (5 mL). Pd/C (10%, 20 mg) was added to the THF solution. The mixture underwent hydrogenation at RT overnight until


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LC−MS indicated that the reaction was complete. The filtrate was concentrated and the residue was purified by preparative HPLC to afford the title compound as a pale yellow solid (8 mg, 29% yield for two steps). $^1$H NMR (300 MHz, $CD_3OD$) $\delta$ ppm 8.37 (s, 1H), 8.32 (d, $J$ = 8.1 Hz, 1H), 7.74 (d, $J$ = 1.3 Hz, 1H), 7.56−7.39(m, 2H), 5.39−5.32 (m, 1H), 3.71−3.68 (m, 2H), 3.55−3.37 (m, 4H), 3.29−3.22(m, 2H), 3.05 (s, 3H), 2.90−2.70 (m, 2H), 1.87 (d, $J$ = 7.1 Hz, 6H), 1.85−1.73(m, 2H), 1.04 (t, $J$ = 7.3 Hz, 3H).

**2-Isobutyl-5-isopropyl-3-(4-methylpiperazin-1-yl)-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (8d).** A mixture of 2-bromo-5-isopropyl-3-(4-methylpiperazin-1-yl)-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (30 mg, 0.061 mmol), $Na_2CO_3$ (25 mg, 0.183 mmol), $Pd(PPh_3)_2Cl_2$ (4.3 mg, 0.0061 mmol), and 4,4,5,5-tetramethyl-2-(2-methylprop-1-en-1-yl)-1,3,2-dioxaborolane (16 mg, 0.091 mmol) in DME/$H_2O$ (4.8 mL, 5/1, v/v) was stirred under nitrogen at 80 °C overnight. After the reaction was complete as indicated by LC−MS, the mixture was purified by preparative HPLC to afford 5-isopropyl-3-(4-methylpiperazin-1-yl)-2-(2-methylprop-1-en-1-yl)-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]-quinoline-8-carbonitrile, which was dissolved in THF (5 mL). Pd/C (10%, 20 mg) was added to the THF solution. The mixture underwent hydrogenation at RT overnight until LC−MS indicated that the reaction was complete. The filtrate was concentrated and the residue was purified by preparative HPLC to afford the title compound as a pale yellow solid (10 mg, 35% yield for two steps). $^1$H NMR (300 MHz, $CD_3OD$) $\delta$ ppm 8.45 (s, 1H), 8.22 (d, $J$ = 8.0 Hz, 1H), 7.62 (s, 1H), 7.39 (s, 1H), 7.18 (d, $J$ = 8.0 Hz, 1H), 5.50−5.40 (m, 1H), 3.70−3.60 (m, 2H), 3.55−3.30 (m, 6H), 2.75 (d, $J$ = 7.1 Hz, 2H), 2.53 (s, 3H), 2.25−2.15 (m, 1H), 1.90 (d, $J$ = 7.1 Hz, 6H), 0.99 (d, $J$ = 6.6 Hz, 6H).

**2,5-Diisopropyl-3-(4-methylpiperazin-1-yl)-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (8e).** A mixture of 2-bromo-5-isopropyl-3-(4-methylpiperazin-1-yl)-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (30 mg, 0.061 mmol), $Na_2CO_3$ (25 mg, 0.183 mmol), $Pd(PPh_3)_2Cl_2$ (4.3 mg, 0.0061 mmol), and 4,4,5,5-tetramethyl-2-(prop-1-en-2-yl)-1,3,2-dioxaborolane (16 mg, 0.091 mmol) in DME/$H_2O$ (4.8 mL, 5/1, v/v) was stirred under nitrogen at 80 °C overnight. After the reaction was complete as indicated by LC−MS, the mixture was purified by preparative HPLC to afford 5-isopropyl-3-(4-methylpiperazin-1-yl)-11-oxo-2-(prop-1-en-2-yl)-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile, which was dissolved in THF (5 mL). Pd/C (10%; 20 mg) was added to the THF solution. The resulting mixture underwent hydrogenation at RT overnight until LC−MS indicated that the reaction was complete. The filtrate after filtration was concentrated and the residue was purified by preparative HPLC to afford the title compound as a pale yellow solid (9 mg, 32% yield for two steps). $^1$H NMR (300 MHz, $CD_3OD$) $\delta$ ppm 8.53 (s, 1H), 8.42 (d, $J$ = 8.0 Hz, 1H), 7.82 (m, 1H), 7.60−7.52 (m, 2H), 5.45−5.27 (m, 1H), 3.92−3.25 (m, 9H), 3.07 (s, 3H), 1.89 (d, $J$ = 6.1 Hz, 6H), 1.38 (d, $J$ = 6.8 Hz, 6H).

**Methyl 6-Cyano-2-((3-fluoro-4-methoxyphenyl)(isopropyl)-amino)-1*H*-indole-3-carboxylate (17a).** DABCO (62 mg, 0.55 mmol) was added to a solution of methyl 6-cyano-1*H*-indole-3-carboxylate (200 mg, 1 mmol) in DCM (20 mL) and the mixture was cooled down to 0 °C. NCS (147 mg, 1.1 mmol) was then added and the reaction was stirred at 0 °C for 2 h. A solution of 3-fluoro-*N*-isopropyl-4-methoxyaniline (183 mg, 1 mmol) and trichloroacetic acid (41 mg, 0.25 mmol) in DCM (5 mL) was added dropwise and the reaction was stirred for 2 h at RT. The reaction was quenched by adding $H_2O$ and the mixture was extracted with DCM. The solvent was removed under vacuum and the residue was purified by silica gel chromatography with hexane/EtOAc (4/1, v/v) to afford the title compound as a colorless oil (250 mg, 66% yield). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ ppm 8.08−8.06 (m, 2H), 7.45−7.36 (m, 2H), 7.05−6.87 (m, 3H), 4.88−4.80 (m, 1H), 3.92 (s, 3H), 3.88 (s, 3H), 1.26 (d, $J$ = 6.6 Hz, 6H).

**Methyl 6-Cyano-2-((3-fluoro-4-propoxyphenyl)(isopropyl)-amino)-1*H*-indole-3-carboxylate (17b).** DABCO (62 mg, 0.55 mmol) was added to a solution of methyl 6-cyano-1*H*-indole-3-carboxylate (200 mg, 1 mmol) in DCM (20 mL) and the mixture was cooled down to 0 °C. NCS (147 mg, 1.1 mmol) was then added and the reaction was stirred at 0 °C for 2 h. A solution of 3-fluoro-*N*-isopropyl-4-propoxyaniline (211 mg, 1 mmol) and trichloroacetic acid (41 mg, 0.25 mmol) in DCM (5 mL) was added dropwise and the reaction was stirred for 2 h at RT. The reaction was quenched by adding $H_2O$ and the mixture was extracted with DCM. Solvent was removed under vacuum and the residue was purified by silica gel chromatography with hexane/EtOAc (4/1, v/v) to afford the title compound as a colorless oil (235 mg, 57% yield). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ ppm 8.05 (d, $J$ = 8.3 Hz, 1H), 7.95 (s, 1H), 7.41 (dd, $J$ = 8.3, 1.4 Hz, 1H), 7.37 (d, $J$ = 1.4 Hz, 1H), 7.01 (t, $J$ = 8.9 Hz, 1H), 6.97 (dd, $J$ = 12.0, 2.6 Hz, 1H), 6.90−6.85 (m, 1H), 4.91−4.82 (m, 1H), 4.03 (t, $J$ = 6.6 Hz, 2H), 3.89 (s, 3H), 1.90−1.88 (m, 2H), 1.26 (d, $J$ = 6.6 Hz, 6H), 1.09 (t, $J$ = 7.4 Hz, 3H).

**Methyl 2-((4-Butoxy-3-fluorophenyl)(isopropyl)amino)-6-cyano-1*H*-indole-3-carboxylate (17c).** DABCO (62 mg, 0.55 mmol) was added to a solution of methyl 6-cyano-1*H*-indole-3-carboxylate (200 mg, 1 mmol) in DCM (20 mL) and the mixture was cooled down to 0 °C. NCS (147 mg, 1.1 mmol) was then added and the reaction was stirred at 0 °C for 2 h. A solution of 4-butoxy-3-fluoro-*N*-isopropylaniline (225 mg, 1 mmol) and trichloroacetic acid (41 mg, 0.25 mmol) in DCM (5 mL) was added dropwise and the reaction was stirred for 2 h at RT. The reaction was quenched by adding $H_2O$ and the mixture was extracted with DCM. Solvent was removed under vacuum and the residue was purified by silica gel chromatography with hexane/EtOAc (4/1, v/v) to afford the title compound as a colorless oil (245 mg, 58% yield). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ ppm 8.05 (d, $J$ = 8.3 Hz, 1H), 7.86 (s, 1H), 7.41 (dd, $J$ = 8.3, 1.4 Hz, 1H), 7.37 (d, $J$ = 1.4 Hz, 1H), 7.01 (t, $J$ = 8.9 Hz, 1H), 6.97 (dd, $J$ = 12.1, 2.6 Hz, 1H), 6.91−6.88 (m, 1H), 4.91−4.83 (m, 1H), 4.08 (t, $J$ = 6.5 Hz, 2H), 3.90 (s, 3H), 1.87−1.83 (m, 2H), 1.58−1.48 (m, 2H), 1.27 (d, $J$ = 6.7 Hz, 6H), 1.02 (t, $J$ = 7.4 Hz, 3H).

**Methyl 6-Cyano-2-((3-fluoro-4-isopropoxyphenyl)-(isopropyl)amino)-1*H*-indole-3-carboxylate (17d).** DABCO (62 mg, 0.55 mmol) was added to a solution of methyl 6-cyano-1*H*-indole-3-carboxylate (200 mg, 1 mmol) in DCM (20 mL) and the mixture was cooled down to 0 °C. NCS (147 mg, 1.1 mmol) was then added and the reaction was stirred at 0 °C for 2 h. A solution of 3-fluoro-4-isopropoxy-*N*-isopropylaniline (211 mg, 1 mmol) and trichloroacetic acid (41 mg, 0.25 mmol) in DCM (5 mL) was added dropwise and the reaction was stirred for 2 h at RT. The reaction was quenched by adding $H_2O$ and the mixture was extracted with DCM. Solvent was removed under vacuum and the residue was purified by silica gel chromatography with hexane/EtOAc (4/1, v/v) to afford the title compound as a colorless oil (220 mg, 54% yield). $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ ppm 8.05 (d, $J$ = 8.3 Hz, 1H), 7.85 (s, 1H), 7.45−7.33 (m, 2H), 6.99 (t, $J$ = 8.9 Hz, 1H), 6.91 (dd, $J$ = 12.1, 2.7 Hz, 1H), 6.82−6.80 (m, 1H), 4.81 (m, 1H), 4.54 (m, 1H), 3.86 (s, 3H), 1.39 (d, $J$ = 6.1 Hz, 6H), 1.31−1.21 (m, 6H).

**3-Fluoro-5-isopropyl-2-methoxy-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (18a).** Methyl 6-cyano-2-((3-fluoro-4-methoxyphenyl)(isopropyl)amino)-1*H*-indole-3-carboxylate (250 mg, 0.66 mmol) was dissolved in diphenyl ether (10 mL) and the solution was refluxed for 1 h. The mixture was cooled down to RT. Hexane (20 mL) was added and the precipitate was collected by filtration. The precipitate was purified by silica gel chromatography with hexane/EtOAc (2/1, v/v) to afford the title compound as a pale yellow solid (175 mg, 76% yield). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 12.15 (s, 1H), 8.32 (d, $J$ = 8.0 Hz, 1H), 8.04−7.94 (m, 2H), 7.90 (d, $J$ = 1.3 Hz, 1H), 7.63 (dd, $J$ = 8.0, 1.3 Hz, 1H), 5.35−5.27 (m, 1H), 3.98 (s, 3H), 1.73 (d, $J$ = 6.9 Hz, 6H).

**3-Fluoro-5-isopropyl-11-oxo-2-propoxy-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (18b).** Methyl 6-cyano-2-((3-fluoro-4-propoxyphenyl)(isopropyl)amino)-1*H*-indole-3-carboxylate (235 mg, 0.57 mmol) was dissolved in diphenyl ether (10 mL) and the solution was refluxed for 1 h. The mixture was cooled down to RT. Hexane (20 mL) was added and the precipitate was collected by filtration to afford the title compound as a pale yellow solid (130 mg, 60% yield). This product was used directly in the next step without further purification. MS: $m/z$ = 378 [M + H].

**2-Butoxy-3-fluoro-5-isopropyl-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (18c).** Methyl 2-((4-butoxy-3-fluorophenyl)(isopropyl)amino)-6-cyano-1*H*-indole-3-carboxylate (245 mg, 0.58 mmol) was dissolved in diphenyl ether (10 mL) and


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the solution was refluxed for 1 h. The mixture was cooled down to RT. Hexane (20 mL) was added and the precipitate was collected by filtration to afford the title compound as a pale yellow solid (165 mg, 73% yield). This product was used directly in the next step without further purification. MS: $m/z$ = 392 [M + H].

**3-Fluoro-2-isopropoxy-5-isopropyl-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (18d).** Methyl 6-cyano-2-((3-fluoro-4-isopropoxyphenyl)(isopropyl)amino)-1*H*-indole-3-carboxylate (220 mg, 0.54 mmol) was dissolved in diphenyl ether (10 mL) and the solution was refluxed for 1 h. The mixture was cooled down to RT. Hexane (20 mL) was added and the precipitate was collected by filtration to afford the title compound as a pale yellow solid (130 mg, 70% yield). This product was used directly in the next step without further purification. MS: $m/z$ = 378 [M + H].

**5-Isopropyl-2-methoxy-3-(4-methylpiperazin-1-yl)-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (8f).** 1-Methylpiperazine (0.5 mL) and DIPEA (0.5 mL) were added to a solution of 3-fluoro-5-isopropyl-2-methoxy-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (175 mg, 0.50 mmol) in DMSO (2 mL) and the mixture was heated to 120−140 °C for 3 days. The reaction mixture was cooled down to RT and purified by preparative HPLC to afford the title compound as a pale yellow solid (56 mg, 26% yield). $^1$H NMR (300 MHz, $CD_3OD$) $\delta$ ppm 8.42 (d, $J$ = 8.1 Hz, 1H), 8.00 (s, 1H), 7.82 (s, 1H), 7.57 (d, $J$ = 8.1 Hz, 1H), 7.36 (s, 1H), 5.44−5.35 (m, 1H), 4.05 (s, 3H), 4.02−3.92 (m, 2H), 3.70−3.63 (m, 2H), 3.50−3.18 (m, 4H), 3.05 (s, 3H), 1.88 (d, $J$ = 7.1 Hz, 6H).

**5-Isopropyl-3-(4-methylpiperazin-1-yl)-11-oxo-2-propoxy-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (8g).** 1-Methylpiperazine (0.5 mL) and DIPEA (0.5 mL) were added to a solution of 3-fluoro-5-isopropyl-11-oxo-2-propoxy-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (130 mg, 0.34 mmol) in DMSO (2 mL) and the mixture was heated to 120−140 °C for 3 days. The reaction mixture was cooled down to RT and purified by preparative HPLC to afford the title compound as a pale yellow solid (28 mg, 18% yield). $^1$H NMR (300 MHz, $CD_3OD$) $\delta$ ppm 8.37 (d, $J$ = 8.1 Hz, 1H), 7.93 (s, 1H), 7.78 (s, 1H), 7.54 (d, $J$ = 8.1 Hz, 1H), 7.33 (s, 1H), 5.46−5.25 (m, 1H), 4.18 (t, $J$ = 6.4 Hz, 2H), 4.10−3.97 (m, 2H), 3.75−3.65 (m, 2H), 3.50−3.20 (m, 4H), 3.06 (s, 3H), 2.03−1.94 (m, 2H), 1.88 (d, $J$ = 7.1 Hz, 6H), 1.17 (t, $J$ = 7.4 Hz, 3H).

**2-Butoxy-5-isopropyl-3-(4-methylpiperazin-1-yl)-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (8h).** 1-Methylpiperazine (0.5 mL) and DIPEA (0.5 mL) were added to a solution of 2-butoxy-3-fluoro-5-isopropyl-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (165 mg, 0.42 mmol) in DMSO (2 mL) and the mixture was heated to 120−140 °C for 3 days. The reaction mixture was cooled down to RT and purified by preparative HPLC to afford the title compound as a pale yellow solid (36 mg, 18% yield). $^1$H NMR (300 MHz, $CD_3OD$) $\delta$ ppm 8.39 (d, $J$ = 8.1 Hz, 1H), 7.95 (s, 1H), 7.79 (d, $J$ = 1.4 Hz, 1H), 7.55 (dd, $J$ = 8.1, 1.4 Hz, 1H), 7.32 (s, 1H), 5.40−5.32 (m, 1H), 4.21 (t, $J$ = 6.4 Hz, 2H), 3.99−3.95 (m, 2H), 3.71−3.69 (m, 2H), 3.43−3.40 (m, 2H), 3.28−3.12 (m, 2H), 3.04 (s, 3H), 1.96−1.89 (m, 2H), 1.87 (d, $J$ = 7.1 Hz, 6H), 1.68−1.54 (m, 2H), 1.06 (t, $J$ = 7.4 Hz, 3H).

**2-Isopropoxy-5-isopropyl-3-(4-methylpiperazin-1-yl)-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (8i).** 1-Methylpiperazine (0.5 mL) and DIPEA (0.5 mL) were added to a solution of 3-fluoro-2-isopropoxy-5-isopropyl-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (130 mg, 0.34 mmol) in DMSO (2 mL) and the mixture was heated to 120−140 °C for 3 days. The reaction mixture was cooled down to RT and purified by preparative HPLC to afford the title compound as a pale yellow solid (35 mg, 22% yield). $^1$H NMR (300 MHz, $CD_3OD$) $\delta$ ppm 8.43 (d, $J$ = 8.6 Hz, 1H), 8.01 (s, 1H), 7.84 (s, 1H), 7.59 (d, $J$ = 8.6 Hz, 1H), 7.36 (s, 1H), 5.42−5.35 (m, 1H), 4.90−4.80 (m, 1H), 4.02−3.95 (m, 2H), 3.70−3.63 (m, 2H), 3.50−3.10 (m, 4H), 3.05 (s, 3H), 1.88 (d, $J$ = 7.1 Hz, 6H), 1.47 (d, $J$ = 6.0 Hz, 6H).

**Methyl 6-Cyano-2-((2,3-difluorophenyl)(isopropyl)amino)-1*H*-indole-3-carboxylate (19a).** DABCO (62 mg, 0.55 mmol) was added to a solution of methyl 6-cyano-1*H*-indole-3-carboxylate (200 mg, 1 mmol) in DCM (20 mL) and the mixture was cooled down to 0 °C. NCS (147 mg, 1.1 mmol) was then added and the reaction was stirred at 0 °C for 2 h. A solution of 2,3-difluoro-*N*-isopropylaniline (171 mg, 1 mmol) and trichloroacetic acid (41 mg, 0.25 mmol) in DCM (5 mL) was added dropwise and the reaction was stirred for 2 h at RT. The reaction was quenched by adding $H_2O$ and the mixture was extracted with DCM. Solvent was removed under vacuum and the residue was purified by silica gel chromatography with hexane/EtOAc (4/1, v/v) to afford the title compound as a colorless oil (200 mg, 54% yield). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ ppm 8.18 (s, 1H), 8.09 (d, $J$ = 8.4 Hz, 1H), 7.51 (d, $J$ = 1.4 Hz, 1H), 7.45 (dd, $J$ = 8.4, 1.4 Hz, 1H), 7.17−7.07 (m, 3H), 4.77−4.61 (m, 1H), 3.82 (s, 3H), 1.34 (d, $J$ = 6.6 Hz, 6H).

**Methyl 6-Cyano-2-(isopropyl(2,3,4-trifluorophenyl)amino)-1*H*-indole-3-carboxylate (19b).** DABCO (62 mg, 0.55 mmol) was added to a solution of methyl 6-cyano-1*H*-indole-3-carboxylate (200 mg, 1 mmol) in DCM (20 mL) and the mixture was cooled down to 0 °C. NCS (147 mg, 1.1 mmol) was then added and the reaction was stirred at 0 °C for 2 h. A solution of 2,3,4-trifluoro-*N*-isopropylaniline (189 mg, 1 mmol) and trichloroacetic acid (41 mg, 0.25 mmol) in DCM (5 mL) were added dropwise and the reaction was stirred for 2 h at RT. The reaction was quenched by adding $H_2O$ and the mixture was extracted with DCM. Solvent was removed under vacuum and the residue was purified by silica gel chromatography with hexane/EtOAc (4/1, v/v) to afford the title compound as a colorless oil (210 mg, 54% yield). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ ppm 8.36 (s, 1H), 8.07 (d, $J$ = 8.3 Hz, 1H), 7.53 (d, $J$ = 1.4 Hz, 1H), 7.44 (dd, $J$ = 8.3, 1.4 Hz, 1H), 7.15−7.10 (m, 1H), 7.05−7.00 (m, 1H), 4.65−4.60 (m, 1H), 3.82 (s, 3H), 1.33 (dd, $J$ = 6.5, 0.9 Hz, 6H).

**Methyl 6-Cyano-2-((2,3-difluoro-4-methoxyphenyl)(isopropyl)amino)-1*H*-indole-3-carboxylate (19c).** DABCO (62 mg, 0.55 mmol) was added to a solution of methyl 6-cyano-1*H*-indole-3-carboxylate (200 mg, 1 mmol) in DCM (20 mL) and the mixture was cooled down to 0 °C. NCS (147 mg, 1.1 mmol) was then added and the reaction was stirred at 0 °C for 2 h. A solution of 2,3-difluoro-*N*-isopropyl-4-methoxyaniline (201 mg, 1 mmol) and trichloroacetic acid (41 mg, 0.25 mmol) in DCM (5 mL) were added dropwise and the reaction was stirred for 2 h at RT. The reaction was quenched by adding $H_2O$ and the mixture was extracted with DCM. The solvent was removed under vacuum and the residue was purified by silica gel chromatography with hexane/EtOAc (4/1, v/v) to afford the title compound as a colorless oil (225 mg, 56% yield). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ ppm 8.25 (s, 1H), 8.02 (d, $J$ = 8.0 Hz, 1H), 7.45−7.36 (m, 2H), 7.13−7.06 (m, 1H), 6.846.80 (m, 1H), 4.85−4.79 (m, 1H), 3.95 (s, 3H), 3.85 (s, 3H), 1.27 (dd, $J$ = 6.5, 1.1 Hz, 6H).

**3,4-Difluoro-5-isopropyl-11-oxo-6,11-dihydro-5*H*-indolo-[2,3-*b*]quinoline-8-carbonitrile (20a).** Methyl 6-cyano-2-((2,3-difluorophenyl)(isopropyl)amino)-1*H*-indole-3-carboxylate (200 mg, 0.54 mmol) was dissolved in diphenyl ether (10 mL) and the solution was refluxed for 1 h. The mixture was cooled down to RT. Hexane (20 mL) was added and the precipitate was collected by filtration to afford the title compound as a pale yellow solid (112 mg, 61% yield). This product was used directly in the next step without further purification. MS: $m/z$ = 338 [M + H].

**2,3,4-Trifluoro-5-isopropyl-11-oxo-6,11-dihydro-5*H*-indolo-[2,3-*b*]quinoline-8-carbonitrile (20b).** Methyl 6-cyano-2-(isopropyl(2,3,4-trifluorophenyl)amino)-1*H*-indole-3-carboxylate (210 mg, 0.54 mmol) was dissolved in diphenyl ether (10 mL) and the solution was refluxed for 1 h. The mixture was cooled down to RT. Hexane (20 mL) was added and the precipitate was collected by filtration to afford the title compound as a pale yellow solid (100 mg, 52% yield). This product was used directly in the next step without further purification. MS: $m/z$ = 356 [M + H].

**3,4-Difluoro-5-isopropyl-2-methoxy-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (20c).** Methyl 6-cyano-2-((2,3-difluoro-4-methoxyphenyl)(isopropyl)amino)-1*H*-indole-3-carboxylate (225 mg, 0.56 mmol) was dissolved in diphenyl ether (10 mL) and the solution was refluxed for 1 h. The mixture was cooled down to RT. Hexane (20 mL) was added and the precipitate was collected by filtration to afford the title compound as a pale yellow solid


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(135 mg, 65% yield). This product was used directly in the next step without further purification. MS: $m/z$ = 368 [M + H].

**4-Fluoro-5-isopropyl-3-(4-methylpiperazin-1-yl)-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (9a).** 1-Methylpiperazine (0.5 mL) and DIPEA (0.5 mL) were added to a solution of 3,4-difluoro-5-isopropyl-11-oxo-6,11-dihydro-5*H*-indolo-[2,3-*b*]quinoline-8-carbonitrile (112 mg, 0.33 mmol) in DMSO (2 mL) and the mixture was heated to 120−140 °C for 3 days. The reaction mixture was cooled down to RT and purified by preparative HPLC to afford the title compound as a pale yellow solid (22 mg, 16% yield). MS: $m/z$ = 418 [M + H].

**2,4-Difluoro-5-isopropyl-3-(4-methylpiperazin-1-yl)-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (9b).** 1-Methylpiperazine (0.5 mL) and DIPEA (0.5 mL) were added to a solution of 2,3,4-trifluoro-5-isopropyl-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (100 mg, 0.28 mmol) in DMSO (2 mL) and the mixture was heated to 120−140 °C for 3 days. The reaction was cooled down to RT and purified by preparative HPLC to afford the title compound as a pale yellow solid (25 mg, 20% yield). MS: $m/z$ = 436 [M + H]. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 12.16 (s, 1H), 8.24 (d, $J$ = 8.0 Hz, 1H), 7.90 (d, $J$ = 1.3 Hz, 1H), 7.82 (dd, $J$ = 11.8, 1.6 Hz, 1H), 7.61 (dd, $J$ = 8.0, 1.3 Hz, 1H), 5.02 (hept, $J$ = 7.0 Hz, 1H), 3.61−3.52 (m, 4H), 3.44−3.24 (m, 4H), 2.91 (s, 3H), 1.66 (dd, $J$ = 7.0, 1.9 Hz, 6H).

**4-Fluoro-5-isopropyl-2-methoxy-3-(4-methylpiperazin-1-yl)-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (9c).** 1-Methylpiperazine (0.5 mL) and DIPEA (0.5 mL) were added to a solution of 3,4-difluoro-5-isopropyl-2-methoxy-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (135 mg, 0.37 mmol) in DMSO (2 mL) and the mixture was heated to 120−140 °C for 3 days. The reaction mixture was cooled down to RT and purified by preparative HPLC to afford the title compound as a pale yellow solid (24 mg, 15% yield). $^1$H NMR (300 MHz, $CD_3OD$ + $CDCl_3$) $\delta$ ppm 8.33 (d, $J$ = 8.0 Hz, 1H), 7.77 (s, 1H), 7.72 (s, 1H), 7.48 (d, $J$ = 8.0 Hz, 1H), 5.20−5.00 (m, 1H), 4.04 (s, 3H), 3.80−3.50 (m, 6H), 3.40−3.25 (m, 2H), 3.00 (s, 3H), 1.74 (dd, $J$ = 5.6, 1.8 Hz, 6H).

**Methyl 6-Cyano-2-(ethyl(3-fluoro-4-methoxyphenyl)-amino)-1*H*-indole-3-carboxylate (23a).** DABCO (62 mg, 0.55 mmol) was added to a solution of methyl 6-cyano-1*H*-indole-3-carboxylate (200 mg, 1 mmol) in DCM (20 mL) and the mixture was cooled down to 0 °C. NCS (147 mg, 1.1 mmol) was then added and the reaction was stirred at 0 °C for 2 h. A solution of *N*-ethyl-3-fluoro-4-methoxyaniline (169 mg, 1 mmol) and trichloroacetic acid (41 mg, 0.25 mmol) in DCM (5 mL) was added dropwise and the reaction was stirred for 2 h at RT. The reaction was quenched by adding $H_2O$ and the mixture was extracted with DCM. The solvent was removed under vacuum and the residue was purified by silica gel chromatography with hexane/EtOAc (4/1, v/v) to afford the title compound as a colorless oil (160 mg, 44% yield). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ ppm 8.31 (s, 1H), 8.09 (d, $J$ = 8.7 Hz, 1H), 7.47−7.39 (m, 2H), 7.02−6.84 (m, 3H), 4.01 (q, $J$ = 7.1 Hz, 2H), 3.89 (s, 3H), 3.87 (s, 3H), 1.30 (t, $J$ = 7.1 Hz, 3H).

**Methyl 6-Cyano-2-((3-fluoro-4-methoxyphenyl)(propyl)-amino)-1*H*-indole-3-carboxylate (23b).** DABCO (62 mg, 0.55 mmol) was added to a solution of methyl 6-cyano-1*H*-indole-3-carboxylate (200 mg, 1 mmol) in DCM (20 mL) and the mixture was cooled down to 0 °C. NCS (147 mg, 1.1 mmol) was then added and the reaction was stirred at 0 °C for 2 h. A solution of *N*-propyl-3-fluoro-4-methoxyaniline (183 mg, 1 mmol) and trichloroacetic acid (41 mg, 0.25 mmol) in DCM (5 mL) was added dropwise and the reaction was stirred for 2 h at RT. The reaction was quenched by adding $H_2O$ and the mixture was extracted with DCM. The solvent was removed under vacuum and the residue was purified by silica gel chromatography with hexane/EtOAc (4/1, v/v) to afford the title compound as a colorless oil (170 mg, 45% yield). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ ppm $\delta$ 8.28 (s, 1H), 8.10 (d, $J$ = 8.7 Hz, 1H), 7.47−7.41 (m, 2H), 7.02−6.79 (m, 3H), 4.00−3.79 (m, 8H), 1.80−1.65 (m, 2H), 0.96 (q, $J$ = 7.3 Hz, 3H).

**Methyl 2-(*sec*-Butyl(3-fluoro-4-methoxyphenyl)amino)-6-cyano-1*H*-indole-3-carboxylate (23c).** DABCO (62 mg, 0.55 mmol) was added to a solution of methyl 6-cyano-1*H*-indole-3-carboxylate (200 mg, 1 mmol) in DCM (20 mL) and the mixture was cooled down to 0 °C. NCS (147 mg, 1.1 mmol) was then added and the reaction was stirred at 0 °C for 2 h. A solution of *N*-(*sec*-butyl)-3-fluoro-4-methoxyaniline (197 mg, 1 mmol) and trichloroacetic acid (41 mg, 0.25 mmol) in DCM (5 mL) was added dropwise and the reaction was stirred for 2 h at RT. The reaction was quenched by adding $H_2O$ and the mixture was extracted with DCM. The solvent was removed under vacuum and the residue was purified by silica gel chromatography with hexane/EtOAc (4/1, v/v) to afford the title compound as a colorless oil (210 mg, 53% yield). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ ppm 8.05 (d, $J$ = 8.3 Hz, 1H), 7.93 (s, 1H), 7.41 (dd, $J$ = 8.3, 1.4 Hz, 1H), 7.37 (d, $J$ = 1.4 Hz, 1H), 7.07−6.85 (m, 3H), 4.60−4.54 (m, 1H), 3.93 (s, 3H), 3.88 (s, 3H), 1.79−1.75 (m, 1H), 1.54−1.51 (m, 1H), 1.26 (d, $J$ = 6.7 Hz, 3H), 0.98 (t, $J$ = 7.4 Hz, 3H).

**Methyl 6-Cyano-2-((3-fluoro-4-methoxyphenyl)(pentan-2-yl)amino)-1*H*-indole-3-carboxylate (23d).** DABCO (62 mg, 0.55 mmol) was added to a solution of methyl 6-cyano-1*H*-indole-3-carboxylate (200 mg, 1 mmol) in DCM (20 mL) and the mixture was cooled down to 0 °C. NCS (147 mg, 1.1 mmol) was then added and the reaction was stirred at 0 °C for 2 h. A solution of 3-fluoro-4-methoxy-*N*-(pentan-2-yl)aniline (211 mg, 1 mmol) and trichloroacetic acid (41 mg, 0.25 mmol) in DCM (5 mL) was added dropwise and the reaction was stirred for 2 h at RT. The reaction was quenched by adding $H_2O$ and the mixture was extracted with DCM. The solvent was removed under vacuum and the residue was purified by silica gel chromatography with hexane/EtOAc (4/1, v/v) to afford the title compound as a colorless oil (196 mg, 48% yield). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ ppm 8.05 (d, $J$ = 8.3 Hz, 1H), 7.77 (br s, 1H), 7.41 (dd, $J$ = 8.3, 1.5 Hz, 1H), 7.36 (d, $J$ = 1.5 Hz, 1H), 7.07−6.90 (m, 3H), 4.78−4.61 (m, 1H), 3.95 (s, 3H), 3.89 (s, 3H), 1.72−1.65 (m, 1H), 1.49−1.41 (m, 3H), 1.26 (d, $J$ = 6.8 Hz, 3H), 0.92 (t, $J$ = 7.2 Hz, 3H).

**Methyl 6-Cyano-2-(cyclopropyl(3-fluoro-4-methoxyphenyl)amino)-1*H*-indole-3-carboxylate (23e).** DABCO (62 mg, 0.55 mmol) was added to a solution of methyl 6-cyano-1*H*-indole-3-carboxylate (200 mg, 1 mmol) in DCM (20 mL) and the mixture was cooled down to 0 °C. *N*-Chlorosuccinimide (147 mg, 1.1 mmol) was then added and the reaction was stirred at 0 °C for 2 h. A solution of *N*-cyclopropyl-3-fluoro-4-methoxyaniline (181 mg, 1 mmol) and trichloroacetic acid (41 mg, 0.25 mmol) in DCM (5 mL) was added dropwise and the reaction was stirred for 2 h at room temperature. The reaction was quenched by adding water and the mixture was extracted with DCM. The solvent was removed under vacuum and the residue was purified by silica gel chromatography (Hex:EA = 4:1) to afford the title compound (180 mg, 47% yield). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 8.94 (s, 1H), 8.17 (d, $J$ = 8.4 Hz, 1H), 7.57 (d, $J$ = 1.4 Hz, 1H), 7.48 (dd, $J$ = 8.4, 1.4 Hz, 1H), 6.94−6.82 (m, 2H), 6.77−6.69 (m, 1H), 3.85 (s, 3H), 3.74 (s, 3H), 3.17−2.99 (m, 1H), 1.00−0.93 (m, 2H), 0.81−0.74 (m, 2H).

**Methyl 6-Cyano-2-(cyclobutyl(3-fluoro-4-methoxyphenyl)-amino)-1*H*-indole-3-carboxylate (23f).** DABCO (62 mg, 0.55 mmol) was added to a solution of methyl 6-cyano-1*H*-indole-3-carboxylate (200 mg, 1 mmol) in DCM (20 mL) and the mixture was cooled down to 0 °C. NCS (147 mg, 1.1 mmol) was then added and the reaction was stirred at 0 °C for 2 h. A solution of *N*-cyclobutyl-3-fluoro-4-methoxyaniline (195 mg, 1 mmol) and trichloroacetic acid (41 mg, 0.25 mmol) in DCM (5 mL) was added dropwise and the reaction was stirred for 2 h at RT. The reaction was quenched by adding $H_2O$ and the mixture was extracted with DCM. The solvent was removed under vacuum and the residue was purified by silica gel chromatography with hexane/EtOAc (4/1, v/v) to afford the title compound as a colorless oil (245 mg, 62% yield). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ ppm 8.62 (s, 1H), 8.17 (d, $J$ = 8.4 Hz, 1H), 7.55 (d, $J$ = 1.5 Hz, 1H), 7.47 (dd, $J$ = 8.4, 1.5 Hz, 1H), 6.89 (t, $J$ = 9.1 Hz, 1H), 6.64 (dd, $J$ = 12.9, 2.8 Hz, 1H), 6.60−6.51 (m, 1H), 4.55−4.43 (m, 1H), 3.86 (s, 3H), 3.81 (s, 3H), 2.40−2.28 (m, 2H), 2.00−1.90 (m, 2H), 1.77−1.63 (m, 2H).

**Methyl 6-Cyano-2-(cyclopentyl(3-fluoro-4-methoxyphenyl)-amino)-1*H*-indole-3-carboxylate (23g).** DABCO (62 mg, 0.55 mmol) was added to a solution of methyl 6-cyano-1*H*-indole-3-carboxylate (200 mg, 1 mmol) in DCM (20 mL) and the mixture was cooled down to 0 °C. NCS (147 mg, 1.1 mmol) was then added and the reaction was stirred at 0 °C for 2 h. A solution of *N*-cyclopentyl-3-fluoro-4-methoxyaniline (209 mg, 1 mmol) and trichloroacetic acid (41


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mg, 0.25 mmol) in DCM (5 mL) was added dropwise and the reaction was stirred for 2 h at RT. The reaction was quenched by adding $H_2O$ and the mixture was extracted with DCM. Solvent was removed under vacuum and the residue was purified by silica gel chromatography with hexane/EtOAc (4/1, v/v) to afford the title compound as a colorless oil (280 mg, 69% yield).$^1$H NMR (400 MHz, $CDCl_3$) $\delta$ ppm 8.55 (s, 1H), 8.13 (d, $J$ = 8.3 Hz, 1H), 7.50 (d, $J$ = 1.4 Hz, 1H), 7.44 (dd, $J$ = 8.3, 1.4 Hz, 1H), 6.92 (t, $J$ = 9.1 Hz, 1H), 6.79 (dd, $J$ = 12.7, 2.7 Hz, 1H), 6.75−6.71 (m, 1H), 4.61−4.52 (m, 1H), 3.87 (s, 3H), 3.83 (s, 3H), 2.09−1.98 (m, 2H), 1.66−1.62 (m, 4H), 1.59−1.47 (m, 2H).

**Methyl 6-Cyano-2-(cyclohexyl(3-fluoro-4-methoxyphenyl)-amino)-1*H*-indole-3-carboxylate (23h).** DABCO (62 mg, 0.55 mmol) was added to a solution of methyl 6-cyano-1*H*-indole-3-carboxylate (200 mg, 1 mmol) in DCM (20 mL) and the mixture was cooled down to 0 °C. NCS (147 mg, 1.1 mmol) was then added and the reaction was stirred at 0 °C for 2 h. A solution of *N*-cyclohexyl-3-fluoro-4-methoxyaniline (223 mg, 1 mmol) and trichloroacetic acid (41 mg, 0.25 mmol) in DCM (5 mL) was added dropwise and the reaction was stirred for 2 h at RT. The reaction was quenched by adding $H_2O$ and the mixture was extracted with DCM. The solvent was removed under vacuum and the residue was purified by silica gel chromatography with hexane/EtOAc (4/1, v/v) to afford the title compound as a colorless oil (240 mg, 57% yield). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ ppm 8.06 (d, $J$ = 8.3 Hz, 1H), 8.02 (s, 1H), 7.45−7.37 (m, 2H), 7.04−6.82 (m, 3H), 4.37−4.26 (m, 1H), 3.92 (s, 3H), 3.88 (s, 3H), 2.10−2.06 (m, 2H), 1.85−1.62 (m, 2H), 1.67−1.63 (m, 2H), 1.47−1.35 (m, 2H), 1.30−1.27 (m, 2H).

**5-Ethyl-3-fluoro-2-methoxy-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (24a).** Methyl 6-cyano-2-(ethyl(3-fluoro-4-methoxyphenyl)amino)-1*H*-indole-3-carboxylate (160 mg, 0.44 mmol) was dissolved in diphenyl ether (10 mL) and the solution was refluxed for 1 h and then cooled down to RT. Hexane (20 mL) was added and the precipitate was collected by filtration. The precipitate was purified by silica gel chromatography with hexane/EtOAc (2/1, v/v) to afford the title compound as a colorless oil (98 mg, 67% yield). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ ppm 12.58 (s, 1H), 8.29 (d, $J$ = 8.1 Hz, 1H), 7.98 (d, $J$ = 9.7 Hz, 1H), 7.94−7.84 (m, 2H), 7.62 (dd, $J$ = 8.1, 1.5 Hz, 1H), 4.52 (d, $J$ = 7.4 Hz, 2H), 3.97 (s, 3H), 1.38 (t, $J$ = 7.4 Hz, 3H).

**3-Fluoro-2-methoxy-11-oxo-5-propyl-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (24b).** Methyl 6-cyano-2-(propyl(3-fluoro-4-methoxyphenyl)amino)-1*H*-indole-3-carboxylate (150 mg, 0.39 mmol) was dissolved in diphenyl ether (10 mL) and the solution was refluxed for 1 h and then cooled down to RT. Hexane (20 mL) was added and the precipitate was collected by filtration. The precipitate was purified by silica gel chromatography with hexane/EtOAc (2/1, v/v) to afford the title compound as a colorless oil (95 mg, 69% yield). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ ppm 12.50 (s, 1H), 8.30 (d, $J$ = 8.0 Hz, 1H), 7.98 (d, $J$ = 9.7 Hz, 1H), 7.93−7.82 (m, 2H), 7.62 (dd, $J$ = 8.0, 1.4 Hz, 1H), 4.41 (t, $J$ = 7.8 Hz, 2H), 3.98 (s, 3H), 1.80 (q, $J$ = 7.8 Hz, 2H), 1.02 (t, $J$ = 7.8 Hz, 3H).

**5-(*sec*-Butyl)-3-fluoro-2-methoxy-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (24c).** Methyl 2-(*sec*-butyl-(3-fluoro-4-methoxyphenyl)amino)-6-cyano-1*H*-indole-3-carboxylate (210 mg, 0.53 mmol) was dissolved in diphenyl ether (10 mL) and the solution was refluxed for 1 h. The mixture was cooled down to RT. Hexane (20 mL) was added and the precipitate was collected by filtration. The precipitate (150 mg, 78% yield) was used directly in the next step without further purification.

**3-Fluoro-2-methoxy-11-oxo-5-(pentan-2-yl)-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (24d).** Methyl 6-cyano-2-((3-fluoro-4-methoxyphenyl)(pentan-2-yl)amino)-1*H*-indole-3-carboxylate (196 mg, 0.48 mmol) was dissolved in diphenyl ether (10 mL) and the solution was refluxed for 1 h. The mixture was cooled down to RT. Hexane (20 mL) was added and the precipitate was collected by filtration. The precipitate was purified by silica gel chromatography with hexane/EtOAc (2/1, v/v) to afford the title compound as a pale yellow solid (148 mg, 82% yield). MS $m/z$ = 378 [M + H]. $^1$H NMR (300 MHz, $CD_3OD$) $\delta$ ppm 8.43 (d, $J$ = 8.0 Hz, 1H), 8.00 (s, 1H), 7.82 (s, 1H), 7.58 (d, $J$ = 8.0 Hz, 1H), 7.36 (s, 1H), 5.05−4.85 (m, 1H), 4.05 (s, 3H), 4.00−3.82 (m, 2H), 3.75−3.60 (m, 2H), 3.50−3.10 (m, 4H), 3.05 (s, 3H), 2.50−2.10 (m, 2H), 1.95−1.75 (m, 3H), 1.50−1.15 (m, 2H), 0.91 (t, $J$ = 7.3 Hz, 3H).

**5-Cyclopropyl-3-fluoro-2-methoxy-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (24e).** Methyl 6-cyano-2-(cyclopropyl(3-fluoro-4-methoxyphenyl)amino)-1*H*-indole-3-carboxylate (180 mg, 0.47 mmol) was dissolved in diphenyl ether (10 mL) and the solution was refluxed for 1 h. The mixture was cooled down to RT. Hexane (20 mL) was added and the precipitate was collected by filtration. The precipitate was purified by silica gel chromatography (Hex:EA = 2:1) to afford the title compound (102 mg, 62% yield). MS $m/z$ = 348 [M + H].

**5-Cyclobutyl-3-fluoro-2-methoxy-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (24f).** Methyl 6-cyano-2-(cyclobutyl(3-fluoro-4-methoxyphenyl)amino)-1*H*-indole-3-carboxylate (245 mg, 0.66 mmol) was dissolved in diphenyl ether (10 mL) and the solution was refluxed for 1 h. The mixture was cooled down to RT. Hexane (20 mL) was added and the precipitate was collected by filtration. The precipitate (155 mg, 65% yield) was used directly in the next step without further purification.

**5-Cyclopentyl-3-fluoro-2-methoxy-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (24g).** Methyl 6-cyano-2-(cyclopentyl(3-fluoro-4-methoxyphenyl)amino)-1*H*-indole-3-carboxylate (280 mg, 0.69 mmol) was dissolved in diphenyl ether (10 mL) and the solution was refluxed for 1 h. The mixture was cooled down to RT. Hexane (20 mL) was added and the precipitate was collected by filtration. The precipitate (130 mg, 50% yield) was used directly in the next step without further purification.

**5-Cyclohexyl-3-fluoro-2-methoxy-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (24h).** Methyl 6-cyano-2-(cyclohexyl(3-fluoro-4-methoxyphenyl)amino)-1*H*-indole-3-carboxylate (240 mg, 0.57 mmol) was dissolved in diphenyl ether (10 mL) and the solution was refluxed for 1 h. The mixture was cooled down to RT. Hexane (20 mL) was added and the precipitate was collected by filtration. The precipitate (160 mg, 72% yield) was used directly in the next step without further purification.

**5-Ethyl-2-methoxy-3-(4-methylpiperazin-1-yl)-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (10a).** 1-Methylpiperazine (0.5 mL) and DIPEA (0.5 mL) were added to a solution of 5-ethyl-3-fluoro-2-methoxy-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (98 mg, 0.29 mmol) in DMSO (2 mL) and the mixture was heated to 120−140 °C for 3 days. The reaction mixture was cooled down to RT and purified by preparative HPLC to afford the title compound as a pale yellow solid (25 mg, 21% yield). $^1$H NMR (300 MHz, $CD_3OD$) $\delta$ ppm 8.31 (d, $J$ = 7.6 Hz, 1H), 7.91 (s, 1H), 7.59 (d, $J$ = 0.7 Hz, 1H), 7.50 (dd, $J$ = 7.6, 0.7 Hz, 1H), 7.06 (s, 1H), 4.50 (q, $J$ = 7.3 Hz, 2H), 4.06 (s, 3H), 4.04−3.86 (m, 2H), 3.75−3.63 (m, 2H), 3.50−3.37 (m, 2H), 3.26−3.17 (m, 2H), 3.05 (s, 3H), 1.53 (t, $J$ = 7.3 Hz, 3H).

**5-Propyl-2-methoxy-3-(4-methylpiperazin-1-yl)-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (10b).** 1-Methylpiperazine (0.5 mL) and DIPEA (0.5 mL) were added to a solution of 5-propyl-3-fluoro-2-methoxy-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (90 mg, 0.26 mmol) in DMSO (2 mL) and the mixture was heated to 120−140 °C for 3 days. The reaction mixture was cooled down to RT and purified by preparative HPLC to afford the title compound as a pale yellow solid (22 mg, 20% yield). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 12.50 (s, 1H), 9.97 (s, 1H), 8.29 (d, $J$ = 8.1 Hz, 1H), 7.92−7.74 (m, 2H), 7.60 (dd, $J$ = 8.1, 1.5 Hz, 1H), 7.09 (s, 1H), 4.49 (t, $J$ = 7.6 Hz, 2H), 3.94 (s, 3H), 3.84 (d, $J$ = 11.8 Hz, 2H), 3.59 (d, $J$ = 11.8 Hz, 2H), 3.33−3.25 (m, 2H), 3.10 (m, 2H), 2.92 (s, 3H), 1.86 (m, 2H), 1.01 (t, $J$ = 7.5 Hz, 3H).

**5-(*sec*-Butyl)-2-methoxy-3-(4-methylpiperazin-1-yl)-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (10c).** 1-Methylpiperazine (0.5 mL) and DIPEA (0.5 mL) were added to a solution of 5-(*sec*-butyl)-3-fluoro-2-methoxy-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (150 mg, 0.41 mmol) in DMSO (2 mL) and the mixture was heated to 120−140 °C for 3 days. The reaction mixture was cooled down to RT and purified by preparative HPLC to afford the title compound as a pale yellow solid (35 mg, 19%


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yield). $^1$H NMR (300 MHz, $CD_3OD$) $\delta$ ppm 8.46 (d, $J$ = 8.1 Hz, 1H), 8.02 (s, 1H), 7.84 (s, 1H), 7.59 (d, $J$ = 8.1, Hz, 1H), 7.36 (s, 1H), 5.05−4.80 (m, 1H), 4.05 (s, 3H), 4.02−3.79 (m, 2H), 3.77−3.61 (m, 2H), 3.51−3.32 (m, 2H), 3.28−3.12 (m, 2H), 3.03 (s, 3H), 2.48−2.40 (m, 1H), 2.25−2.18 (m, 1H), 1.88 (d, $J$ = 6.8 Hz, 3H), 0.90 (t, $J$ = 7.3 Hz, 3H).

**2-Methoxy-3-(4-methylpiperazin-1-yl)-11-oxo-5-(pentan-2-yl)-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (10d).** 1-Methylpiperazine (0.5 mL) and DIPEA (0.5 mL) were added to a solution of 3-fluoro-2-methoxy-11-oxo-5-(pentan-2-yl)-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (148 mg, 0.39 mmol) in DMSO (2 mL) and the mixture was heated to 120−140 °C for 3 days. The reaction was cooled down to RT and purified by preparative HPLC to afford the title compound as a pale yellow solid (45 mg, 23% yield). MS: $m/z$ = 458 [M + H]. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.98 (s, 1H), 8.32 (d, $J$ = 8.0 Hz, 1H), 7.91−7.87 (m, 2H), 7.61 (dd, $J$ = 8.0, 1.4 Hz, 1H), 7.23 (s, 1H), 5.11−5.05 (m, 1H), 3.95 (s, 3H), 3.86−3.77 (m, 2H), 3.59 (d, $J$ = 11.9 Hz, 2H), 3.35−3.23 (m, 2H), 3.12−3.02 (m, 2H), 2.92 (s, 3H), 2.35−2.20 (m, 1H), 2.05−2.01 (m, 1H), 1.76 (d, $J$ = 6.9 Hz, 3H), 1.32−1.25 (m, 1H), 1.11−1.05 (m, 1H), 0.83 (t, $J$ = 7.3 Hz, 3H).

**5-Cyclopropyl-2-methoxy-3-(4-methylpiperazin-1-yl)-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (10e).** 1-Methylpiperazine (0.5 mL) and DIPEA (0.5 mL) were added to a solution of 5-cyclopropyl-3-fluoro-2-methoxy-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (160 mg, 0.41 mmol) in DMSO (2 mL) and the mixture was heated to 120 to 140 °C for 3 days. The reaction was cooled down to RT and purified by preparative HPLC to afford the title compound (36 mg, 18% yield). $^1$H NMR (300 MHz, $CD_3OD$) $\delta$ ppm 8.27 (d, $J$ = 7.8 Hz, 1H), 7.84 (s, 1H), 7.70 (s, 1H), 7.54 (s, 1H), 7.47 (d, $J$ = 7.8 Hz, 1H), 4.04 (s, 3H), 4.03−3.90 (m, 2H), 3.72−3.63 (m, 2H), 3.60−3.37 (m, 3H), 3.25−3.12 (m, 2H), 3.05 (s, 3H), 1.66−1.57 (m, 2H), 1.30−1.18 (m, 2H).

**5-Cyclobutyl-2-methoxy-3-(4-methylpiperazin-1-yl)-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (10f).** 1-Methylpiperazine (0.5 mL) and DIPEA (0.5 mL) were added to a solution of 5-cyclobutyl-3-fluoro-2-methoxy-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (155 mg, 0.50 mmol) in DMSO (2 mL) and the mixture was heated to 120−140 °C for 3 days. The reaction mixture was cooled down to RT and purified by preparative HPLC to afford the title compound as a pale yellow solid (43 mg, 20% yield). $^1$H NMR (300 MHz, $CD_3OD$) $\delta$ ppm 8.39 (d, $J$ = 8.1 Hz, 1H), 7.94 (s, 1H), 7.79 (s, 1H), 7.55 (d, $J$ = 8.1 Hz, 1H), 7.14 (s, 1H), 5.31−5.25 (m, 1H), 4.03 (s, 3H), 3.95−3.91 (m, 2H), 3.69−3.65 (m, 2H), 3.45−3.37 (m, 2H), 3.19−3.14 (m, 2H), 3.07−2.99 (m, 5H), 2.68−2.53 (m, 2H), 2.04−2.02 (m, 2H).

**5-Cyclopentyl-2-methoxy-3-(4-methylpiperazin-1-yl)-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (10g).** 1-Methylpiperazine (0.5 mL) and DIPEA (0.5 mL) were added to a solution of 5-cyclopentyl-3-fluoro-2-methoxy-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (130 mg, 0.35 mmol) in DMSO (2 mL) and the mixture was heated to 120 to 140 °C for 3 days. The reaction mixture was cooled down to RT and purified by preparative HPLC to afford the title compound as a pale yellow solid (51 mg, 32% yield). $^1$H NMR (300 MHz, $CD_3OD$) $\delta$ ppm 8.29 (d, $J$ = 8.1 Hz, 1H), 7.94 (s, 1H), 7.65 (d, $J$ = 1.3 Hz, 1H), 7.45 (dd, $J$ = 8.1, 1.3 Hz, 1H), 7.08 (s, 1H), 5.40−5.28 (m, 1H), 4.03 (s, 3H), 3.96−3.94 (m, 2H), 3.71−3.67 (m, 2H), 3.51−3.18 (m, 4H), 3.03 (s, 3H), 2.54−2.47 (m, 2H), 2.41−2.13 (m, 4H), 2.10−1.89 (m, 2H).

**5-Cyclohexyl-2-methoxy-3-(4-methylpiperazin-1-yl)-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (10h).** 1-Methylpiperazine (0.5 mL) and DIPEA (0.5 mL) were added to a solution of 5-cyclohexyl-3-fluoro-2-methoxy-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (160 mg, 0.41 mmol) in DMSO (2 mL) and the mixture was heated to 120−140 °C for 3 days. The reaction mixture was cooled down to RT and purified by preparative HPLC to afford the title compound as a pale yellow solid (53 mg, 27% yield). $^1$H NMR (300 MHz, $CD_3OD$) $\delta$ ppm 8.42 (d, $J$ = 8.1 Hz, 1H), 7.98 (s, 1H), 7.82 (d, $J$ = 1.4 Hz, 1H), 7.57 (dd, $J$ = 8.1, 1.4 Hz, 1H), 7.42 (s, 1H), 4.90−4.78 (m, 1H),4.03 (s, 3H), 4.00−3.82 (m, 2H), 3.69−3.63 (m, 2H), 3.50−3.37(m, 2H), 3.21−3.11(m, 2H), 3.04 (s, 3H), 2.70−2.49 (m, 2H), 2.11−1.40 (m, 8H).

**(*S*)-4-Fluoro-5-isopropyl-2-methoxy-3-(3-methylpiperazin-1-yl)-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (CJ-2349).** (*S*)-2-Methylpiperazine (0.5 mL) and DIPEA (0.5 mL) were added to a solution of 3,4-difluoro-5-isopropyl-2-methoxy-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (367 mg, 1.0 mmol) in DMSO (3 mL) and the mixture was heated to 120−140 °C for 3 days. The reaction mixture was cooled down to RT and purified by preparative HPLC to afford the title compound (150 mg, 33% yield). $^1$H NMR (400 MHz, $CD_3OD$) $\delta$ 8.36 (d, $J$ = 8.1 Hz, 1H), 7.82−7.76 (m, 2H), 7.55 (d, $J$ = 8.1 Hz, 1H), 5.15−5.08 (m, 1H), 4.04 (s, 3H), 3.65−3.55 (m, 5H), 3.50−3.45 (m, 1H), 3.44−3.35 (m, 1H), 1.80−1.72 (m, 6H), 1.41 (d, $J$ = 6.4 Hz, 3H).

**(*R*)-4-Fluoro-5-isopropyl-2-methoxy-3-(3-methylpiperazin-1-yl)-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (CJ-2350).** (*R*)-2-Methylpiperazine (0.5 mL) and DIPEA (0.5 mL) were added to a solution of 3,4-difluoro-5-isopropyl-2-methoxy-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (367 mg, 1.0 mmol) in DMSO (3 mL) and the mixture was heated to 120−140 °C for 3 days. The reaction mixture was cooled down to RT and purified by preparative HPLC to afford the title compound (132 mg, 30% yield). $^1$H NMR (400 MHz, $CD_3OD$) $\delta$ 8.36 (d, $J$ = 8.1 Hz, 1H), 7.82−7.76 (m, 2H), 7.55 (d, $J$ = 8.1 Hz, 1H), 5.15−5.08 (m, 1H), 4.04 (s, 3H), 3.65−3.55 (m, 5H), 3.50−3.45 (m, 1H), 3.44−3.35 (m, 1H), 1.80−1.72 (m, 6H), 1.41 (d, $J$ = 6.4 Hz, 3H).

**3-(3,3-Dimethylpiperazin-1-yl)-4-fluoro-5-isopropyl-2-methoxy-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (CJ-2351).** 2,2-Dimethylpiperazine (0.5 mL) and DIPEA (0.5 mL) were added to a solution of 3,4-difluoro-5-isopropyl-2-methoxy-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (367 mg, 1.0 mmol) in DMSO (3 mL) and the mixture was heated to 120−140 °C for 3 days. The reaction mixture was cooled down to RT and purified by preparative HPLC to afford the title compound (200 mg, 43% yield). $^1$H NMR (300 MHz, $CD_3OD$) $\delta$ 8.34 (d, $J$ = 7.8 Hz, 1H), 7.80−7.76 (m, 2H), 7.53 (d, $J$ = 7.8 Hz, 1H), 5.15−5.05 (m, 1H), 4.05 (s, 3H), 3.61−3.36 (m, 6H), 1.75 (d, $J$ = 6.7 Hz, 6H), 1.56 (s, 6H).

**(*S*)-3-(3,4-Dimethylpiperazin-1-yl)-4-fluoro-5-isopropyl-2-methoxy-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (CJ-2356).** Formaldehyde solution (37%; 33 mg, 0.40 mmol), acetic acid (12 mg, 0.20 mmol), and sodium triacetoxyborohydride (43 mg, 0.20 mmol) were added to a solution of (*S*)-4-fluoro-5-isopropyl-2-methoxy-3-(3-methylpiperazin-1-yl)-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (60 mg, 0.134 mmol) in DCM (10 mL) and the mixture was stirred at RT for 12 h. Water was added to quench the reaction and the mixture was extracted with DCM. The solvent was removed under vacuum and the residue was purified by preparative HPLC to afford the title compound (40 mg, 65% yield). $^1$H NMR (400 MHz, $CD_3OD$) $\delta$ 8.32 (d, $J$ = 8.0 Hz, 1H), 7.84−7.72 (m, 2H), 7.50 (d, $J$ = 8.0 Hz, 1H), 5.20−5.01 (m, 1H), 4.04 (s, 3H), 3.71−3.59 (m, 5H), 3.50−3.44 (m, 1H), 3.40−3.34 (m, 1H), 3.05 (s, 3H), 1.75 (d, $J$ = 6.8 Hz, 6H), 1.47 (d, $J$ = 6.2 Hz, 3H).

**(*R*)-3-(3,4-Dimethylpiperazin-1-yl)-4-fluoro-5-isopropyl-2-methoxy-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (CJ-2357).** Formaldehyde solution (37%; 33 mg, 0.40 mmol), acetic acid (12 mg, 0.20 mmol), and sodium triacetoxyborohydride (43 mg, 0.20 mmol) were added to a solution of (*R*)-4-fluoro-5-isopropyl-2-methoxy-3-(3-methylpiperazin-1-yl)-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (60 mg, 0.134 mmol) in DCM (10 mL) and the mixture was stirred at RT for 12 h. Water was added to quench the reaction and the mixture was extracted with DCM. The solvent was removed under vacuum and the residue was purified by preparative HPLC to afford the title compound (47 mg, 76% yield). $^1$H NMR (400 MHz, $CD_3OD$) $\delta$ 8.32 (d, $J$ = 8.0 Hz, 1H), 7.84−7.72 (m, 2H), 7.50 (d, $J$ = 8.0 Hz, 1H), 5.20−5.01 (m, 1H), 4.04 (s, 3H), 3.71−3.59 (m, 5H), 3.50−3.44 (m, 1H), 3.40−3.34 (m, 1H), 3.05 (s, 3H), 1.75 (d, $J$ = 6.8 Hz, 6H), 1.47 (d, $J$ = 6.2 Hz, 3H).

**4-Fluoro-5-isopropyl-2-methoxy-11-oxo-3-(3,3,4-trimethylpiperazin-1-yl)-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (CJ-2358).** Formaldehyde solution (37%; 175 mg, 2.17 mmol), acetic acid (39 mg, 0.65 mmol), and sodium triacetoxybor-


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ohydride (138 mg, 0.65 mmol) were added to a solution of 3-(3,3-dimethylpiperazin-1-yl)-4-fluoro-5-isopropyl-2-methoxy-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (200 mg, 0.43 mmol) in DCM (10 mL) and the mixture was stirred at RT for 12 h. Water was added to quench the reaction and the mixture was extracted with DCM. The solvent was removed under vacuum and the residue was purified by preparative HPLC to afford the title compound (150 mg, 73% yield). $^1$H NMR (300 MHz, $CD_3OD$ + $CDCl_3$) $\delta$ 8.39 (d, $J$ = 8.0 Hz, 1H), 7.81−7.75 (m, 2H), 7.53 (d, $J$ = 8.0 Hz, 1H), 5.19−4.99 (m, 1H), 4.04 (s, 3H), 3.66−3.35 (m, 6H), 2.88 (s, 3H), 1.82−1.70 (m, 6H), 1.63 (s, 3H), 1.50 (s, 3H).

**3-(*cis*-3,5-Dimethylpiperazin-1-yl)-4-fluoro-5-isopropyl-2-methoxy-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (CJ-2359).** *cis*-2,6-Dimethylpiperazine (0.5 mL) and DIPEA (0.5 mL) were added to a solution of 3,4-difluoro-5-isopropyl-2-methoxy-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (300 mg, 0.817 mmol) in DMSO (3 mL) and the mixture was heated to 120−140 °C for 3 days. The reaction mixture was cooled down to RT and purified by preparative HPLC to afford the title compound (162 mg, 43% yield). $^1$H NMR (300 MHz, $CD_3OD$) $\delta$ 8.39 (d, $J$ = 8.1 Hz, 1H), 7.88−7.82 (m, 2H), 7.59 (d, $J$ = 8.1 Hz, 1H), 5.15−5.01 (m, 1H), 4.04 (s, 3H), 3.70−3.50 (m, 4H), 3.40−3.32 (m, 2H), 1.76 (d, $J$ = 6.5 Hz, 6H), 1.39 (d, $J$ = 6.3 Hz, 6H).

**4-Fluoro-5-isopropyl-2-methoxy-11-oxo-3-(*cis*-3,4,5-trimethylpiperazin-1-yl)-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (CJ-2360).** Formaldehyde solution (37%; 175 mg, 2.17 mmol), acetic acid (39 mg, 0.65 mmol), and sodium triacetoxyborohydride (138 mg, 0.65 mmol) were added to a solution of 3-(*cis*-3,5-dimethylpiperazin-1-yl)-4-fluoro-5-isopropyl-2-methoxy-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (200 mg, 0.43 mmol) in DCM (10 mL) and methanol (2 mL) and the mixture was stirred at RT for 12 h. Water was added to quench the reaction and the mixture was extracted with DCM. The solvent was removed under vacuum and the residue was purified by preparative HPLC to afford the title compound (135 mg, 66% yield). Purity by HPLC >99%. $^1$H NMR (400 MHz, $CD_3OD$) $\delta$ 8.33 (d, $J$ = 8.1 Hz, 1H), 7.87−7.70 (m, 2H), 7.52 (d, $J$ = 8.1 Hz, 1H), 5.25−5.10 (m, 1H), 4.05 (s, 3H), 3.66−3.40 (m, 6H), 3.06 (s, 3H), 1.72 (d, $J$ = 7.1 Hz, 6H), 1.49 (d, $J$ = 6.0 Hz, 6H). $^{13}$C NMR (101 MHz, $CD_3OD$) $\delta$ 172.14, 151.29 (d, $J_{C-F}$ = 5 Hz), 148.37, 148.16 (d, $J_{C-F}$ = 245 Hz), 134.22, 130.56 (d, $J_{C-F}$ = 13 Hz), 126.29, 125.68 (d, $J_{C-F}$ = 8 Hz), 124.56, 122.86, 120.47, 119.58, 114.18, 104.95, 103.67, 101.40, 61.81, 56.42, 56.31, 55.26, 54.89, 36.01, 19.43, 19.39, 13.59. MS $m/z$ = 476 [M + H].

**2-Bromo-3-fluoro-1-methoxy-4-nitrobenzene (26).** Sodium methoxide (25%; 0.9 g, 4.2 mmol) was added dropwise to a solution of 2-bromo-1,3-difluoro-4-nitrobenzene (1 g, 4.2 mmol) in MeOH (5 mL) and the mixture was stirred at 0 °C for 1 h and RT for 4 h. The reaction was quenched by addition of water and the product was extracted with EtOAc. Solvent was removed under reduced pressure and the residue was purified by silica gel chromatography (Hex:EA = 10:1) to afford the title compound (0.58 g, 55% yield). $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ ppm 8.14 (dd, $J$ = 9.3, 8.3 Hz, 1H), 6.80 (dd, $J$ = 9.3, 1.7 Hz, 1H), 4.03 (s, 3H).

***tert*-Butyl 4-(2-Fluoro-6-methoxy-3-nitrophenyl)-5,6-dihydropyridine-1(2*H*)-carboxylate (27).** *tert*-Butyl 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-5,6-dihydropyridine-1(2*H*)-carboxylate (1.854 g, 6 mmol), Pd(dppf)$Cl_2$ (117 mg, 0.16 mmol), and $K_2CO_3$ (1.656 g, 12 mmol) were added to a solution of 1-bromo-2-iodo-4-nitrobenzene (1 g, 4 mmol) in DME-$H_2O$ (22 mL, 10:1 mixture). The mixture was stirred at 80 °C for 12 h under nitrogen. The reaction was cooled down to RT and the product was extracted with EtOAc. The solvent was removed under reduced pressure and the residue was purified by silica gel chromatography (Hex:EA = 3:1) to afford the title compound (1.3 g, 92% yield). $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ ppm 8.06 (m, 1H), 6.74 (d, $J$ = 9.3 Hz, 1H), 5.72 (s, 1H), 4.15−4.03 (m, 2H), 3.82 (s, 3H), 3.70−3.60 (m, 2H), 2.40−2.30 (m, 2H), 1.50 (s, 9H).

***tert*-Butyl 4-(3-Amino-2-fluoro-6-methoxyphenyl)-piperidine-1-carboxylate (28).** Pd-C (10%; 140 mg) was added to a solution of *tert*-butyl 4-(2-fluoro-6-methoxy-3-nitrophenyl)-5,6-dihydropyridine-1(2*H*)-carboxylate (1.3 g, 3.69 mmol) in ethanol (20 mL) and the mixture underwent hydrogenation at RT overnight. The Pd-C was filtered off. Evaporation of ethanol under reduced pressure afforded the title compound (1.1 g, 92% yield). $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ ppm 6.65−6.45 (m, 2H), 4.30−4.10 (m, 2H), 3.74 (s, 3H), 3.43 (s, 2H), 3.30−3.20 (m, 1H), 2.80−2.70 (m, 2H), 2.20−2.00 (m, 2H), 1.70−1.55 (m, 2H), 1.49 (s, 9H).

***tert*-Butyl 4-(2-Fluoro-3-(isopropylamino)-6-methoxyphenyl)piperidine-1-carboxylate (29).** Acetone (1.74 g, 30 mmol), acetic acid (0.27 g, 4.5 mmol), and sodium triacetoxyborohydride (0.98 g, 4.5 mmol) were added to a solution of *tert*-butyl 4-(3-amino-2-fluoro-6-methoxyphenyl)piperidine-1-carboxylate (1.0 g, 3 mmol) in DCM (20 mL) and the mixture was stirred at RT for 12 h. Water was added to quench the reaction and the mixture was extracted with DCM. The solvent was removed under and the residue was purified by silica gel chromatography to afford the title compound (1.06 g, 94% yield). $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ ppm 6.58−6.44 (m, 2H), 4.30−4.10 (m, 2H), 3.74 (s, 3H), 3.60−3.50 (m, 1H), 3.74 (s, 1H), 3.30−3.17 (m, 1H), 2.83−2.70 (m, 2H), 2.40−2.00 (m, 2H), 1.63−1.55 (m, 2H), 1.49 (s, 9H), 1.21 (d, $J$ = 6.2 Hz, 6H).

**Methyl 2-((3-(1-(*tert*-Butoxycarbonyl)piperidin-4-yl)-2-fluoro-4-methoxyphenyl)(isopropyl)amino)-6-cyano-1*H*-indole-3-carboxylate (30).** DABCO (84 mg, 0.75 mmol) was added to a solution of methyl 6-cyano-1*H*-indole-3-carboxylate (273 mg, 1.366 mmol) in DCM (20 mL) and the mixture was cooled down to 0 °C. *N*-Chlorosuccinimide (200 mg, 1.5 mmol) was then added and the reaction was stirred at 0 °C for 2 h. A solution of *tert*-butyl 4-(2-fluoro-3-(isopropylamino)-6-methoxyphenyl)piperidine-1-carboxylate (500 mg, 1.366 mmol) and trichloroacetic acid (56 mg, 0.342 mmol) in DCM (10 mL) was added dropwise and the reaction was stirred for 2 h at room temperature. The reaction was quenched by adding water and the mixture was extracted with DCM. The solvent was removed under vacuum and the residue was purified by silica gel chromatography (Hex:EA = 4:1) to afford the title compound (530 mg, 69% yield). $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ ppm 8.34 (s, 1H), 7.96 (d, $J$ = 8.2 Hz, 1H), 7.40−7.30 (m, 2H), 7.18 (t, $J$ = 8.7 Hz, 1H), 6.72 (dd, $J$ = 8.9, 1.1 Hz, 1H), 4.90−4.75 (m, 1H), 4.25−4.10 (m, 2H), 3.86 (s, 3H), 3.84 (s, 3H), 3.30−3.20 (m, 1H), 2.80−2.65 (m, 2H), 2.20−2.00 (m, 2H), 1.65−1.50 (m, 2H), 1.46 (s, 9H), 1.20 (d, $J$ = 6.5 Hz, 6H).

**Methyl 6-Cyano-2-((2-fluoro-4-methoxy-3-(1-methylpiperidin-4-yl)phenyl)(isopropyl)amino)-1*H*-indole-3-carboxylate (31b).** TFA (1 mL) was added to a solution of methyl 2-((3-(1-(*tert*-butoxycarbonyl)piperidin-4-yl)-2-fluoro-4-methoxyphenyl)-(isopropyl)amino)-6-cyano-1*H*-indole-3-carboxylate (240 mg, 0.425 mmol) in DCM (5 mL) and the mixture was stirred at RT for 12 h. The solvent was removed under reduced pressure and the residue was neutralized with $NaHCO_3$ and extracted with EtOAc. EtOAc was removed under reduced pressure and the residue was dissolved in DCM (5 mL). HCHO (37%; 100 mg, 1.28 mmol), acetic acid (38 mg, 0.64 mmol), and $NaBH(OAc)_3$ (135 mg, 0.64 mmol) were then added and the reaction mixture was stirred at RT for 6 h. The solvent was removed under reduced pressure and the product was extracted with EtOAc. EtOAc was evaporated under reduced pressure and the residue was purified by silica gel chromatography (EA:MeOH = 80:20) to afford the title compound (180 mg, 89% yield). $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ ppm 8.85 (s, 1H), 7.94 (d, $J$ = 8.3 Hz, 1H), 7.38 (s, 1H), 7.32 (d, $J$ = 8.3 Hz, 1H), 7.15 (t, $J$ = 8.7 Hz, 1H), 6.69 (d, $J$ = 8.7 Hz, 1H), 4.95−4.80 (m, 1H), 3.83 (s, 3H), 3.81 (s, 3H), 3.20−3.00 (m, 3H), 2.45−2.25 (m, 2H), 2.35 (s, 3H), 2.20−2.02 (m, 2H), 1.73−1.60 (m, 2H), 1.19 (d, $J$ = 6.6 Hz, 6H).

**Methyl 6-Cyano-2-((2-fluoro-3-(1-isopropylpiperidin-4-yl)-4-methoxyphenyl)(isopropyl)amino)-1*H*-indole-3-carboxylate (31c).** TFA (1 mL) was added to a solution of methyl 2-((3-(1-(*tert*-butoxycarbonyl)piperidin-4-yl)-2-fluoro-4-methoxyphenyl)-(isopropyl)amino)-6-cyano-1*H*-indole-3-carboxylate (365 mg, 0.647 mmol) in DCM (5 mL) and the mixture was stirred at RT for 12 h. The solvent was removed under reduced pressure and the residue was neutralized with $NaHCO_3$ and extracted with EtOAc. EtOAc was removed under reduced pressure and the residue was dissolved in DCM (5 mL). Acetone (113 mg, 1.94 mmol), acetic acid (58 mg, 0.97 mmol), and $NaBH(OAc)_3$ (206 mg, 0.97 mmol) were then added and the


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reaction mixture was stirred at RT for 12 h. The solvent was removed under reduced pressure and the product was extracted with EtOAc. EtOAc was evaporated under reduced pressure and the residue was purified by silica gel chromatography (EA:MeOH = 80:20) to afford the title compound (150 mg, 46% yield). $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ ppm 7.98−7.90 (m, 2H), 7.35 (dd, $J$ = 8.3, 1.4 Hz, 1H), 7.31 (d, $J$ = 1.4 Hz, 1H), 7.16 (t, $J$ = 8.8 Hz, 1H), 6.72 (d, $J$ = 8.8 Hz, 1H), 4.95−4.80 (m, 1H), 3.87 (s, 6H), 3.20−2.75 (m, 4H), 2.40−2.20 (m, 4H), 1.75−1.55 (m, 2H), 1.19 (d, $J$ = 6.4 Hz, 6H), 1.08 (d, $J$ = 6.5 Hz, 6H).

**Methyl 6-Cyano-2-((2-fluoro-4-methoxy-3-(1-(tetrahydro-2*H*-pyran-4-yl)piperidin-4-yl)phenyl)(isopropyl)amino)-1*H*-indole-3-carboxylate (31d).** TFA (1 mL) was added to a solution of methyl 2-((3-(1-(*tert*-butoxycarbonyl)piperidin-4-yl)-2-fluoro-4-methoxyphenyl)(isopropyl)amino)-6-cyano-1*H*-indole-3-carboxylate (365 mg, 0.647 mmol) in DCM (5 mL) and the mixture was stirred at RT for 12 h. The solvent was removed under reduced pressure and the residue was neutralized with $NaHCO_3$ and extracted with EtOAc. EtOAc was removed under reduced pressure and the residue was dissolved in DCM (5 mL). Dihydro-2*H*-pyran-4(3*H*)-one (194 mg, 1.94 mmol), acetic acid (58 mg, 0.97 mmol), and $NaBH(OAc)_3$ (206 mg, 0.97 mmol) were then added and the reaction mixture was stirred at RT for 12 h. The solvent was removed under reduced pressure and the product was extracted with EtOAc. EtOAc was evaporated under reduced pressure and the residue was purified by silica gel chromatography (EA:MeOH = 80:20) to afford the title compound (140 mg, 39% yield). $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ ppm 8.55 (s, 1H), 7.95 (d, $J$ = 8.3 Hz, 1H), 7.40−7.27 (m, 2H), 7.16 (t, $J$ = 8.7 Hz, 1H), 6.70 (d, $J$ = 8.3 Hz, 1H), 4.95−4.75 (m, 1H), 4.12−4.00 (m, 2H), 3.84 (s, 3H), 3.82 (s, 3H), 3.45−3.25 (m, 2H), 3.15−3.02 (m, 3H), 2.75−2.27 (m, 5H), 1.80−1.60 (m, 6H), 1.20 (d, $J$ = 6.5 Hz, 6H).

**Methyl 6-Cyano-2-((2-fluoro-4-methoxy-3-(1-(oxetan-3-yl)-piperidin-4-yl)phenyl)(isopropyl)amino)-1*H*-indole-3-carboxylate (31e).** TFA (1 mL) was added to a solution of methyl 2-((3-(1-(*tert*-butoxycarbonyl)piperidin-4-yl)-2-fluoro-4-methoxyphenyl)-(isopropyl)amino)-6-cyano-1*H*-indole-3-carboxylate (365 mg, 0.647 mmol) in DCM (5 mL) and the mixture was stirred at RT for 12 h. The solvent was removed under reduced pressure and the residue was neutralized with $NaHCO_3$ and extracted with EtOAc. EtOAc was removed under reduced pressure and the residue was dissolved in DCM (5 mL). Cyclobutanone (140 mg, 1.94 mmol), acetic acid (58 mg, 0.97 mmol), and $NaBH(OAc)_3$ (206 mg, 0.97 mmol) were then added and the reaction mixture was stirred at RT for 6 h. The solvent was removed under reduced pressure and the product was extracted with EtOAc. EtOAc was evaporated under reduced pressure and the residue was purified by silica gel chromatography (EA:MeOH = 80:20) to afford the title compound (190 mg, 58% yield). $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ ppm 8.20 (s, 1H), 7.96 (d, $J$ = 8.2 Hz, 1H), 7.48−7.30 (m, 2H), 7.17 (t, $J$ = 8.7 Hz, 1H), 6.73 (d, $J$ = 8.9 Hz, 1H), 4.95−4.80 (m, 1H), 4.64 (d, $J$ = 6.7 Hz, 4H), 3.88 (s, 3H), 3.85 (s, 3H), 3.51−3.49 (m, 1H), 3.25−3.05 (m, 1H), 2.95−2.75 (m, 2H), 2.45−2.25 (m, 2H), 2.00−1.80 (m, 2H), 1.73−1.60 (m, 2H), 1.20 (d, $J$ = 6.5 Hz, 6H).

**Methyl 2-((3-(1-(2-((*tert*-Butyldimethylsilyl)oxy)ethyl)-piperidin-4-yl)-2-fluoro-4-methoxyphenyl)(isopropyl)amino)-6-cyano-1*H*-indole-3-carboxylate (31f).** TFA (1 mL) was added to a solution of methyl 2-((3-(1-(*tert*-butoxycarbonyl)piperidin-4-yl)-2-fluoro-4-methoxyphenyl)(isopropyl)amino)-6-cyano-1*H*-indole-3-carboxylate (365 mg, 0.647 mmol) in DCM (5 mL) and the mixture was stirred at RT for 12 h. The solvent was removed under reduced pressure and the residue was neutralized with $NaHCO_3$ and extracted with EtOAc. EtOAc was removed under reduced pressure and the residue was dissolved in DCM (5 mL). 2-((*tert*-Butyldimethylsilyl)oxy)-acetaldehyde (164 mg, 0.97 mmol), acetic acid (58 mg, 0.97 mmol), and $NaBH(OAc)_3$ (206 mg, 0.97 mmol) were then added and the reaction mixture was stirred at RT for 6 h. The solvent was removed under reduced pressure and the product was extracted with EtOAc. EtOAc was evaporated under reduced pressure and the residue was purified by silica gel chromatography (EtOAc) to afford the title compound (180 mg, 45% yield). $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ ppm 8.61 (s, 1H), 7.95 (d, $J$ = 8.3 Hz, 1H), 7.40−7.30 (m, 2H), 7.15 (t, $J$ = 8.7 Hz, 1H), 6.69 (d, $J$ = 8.7 Hz, 1H), 4.95−4.78 (m, 1H), 3.90−3.75 (m, 8H), 3.20−3.05 (m, 3H), 2.62 (t, $J$ = 6.3 Hz, 2H), 2.45−2.20 (m, 4H), 1.70−1.55 (m, 2H), 1.20 (d, $J$ = 6.5 Hz, 6H), 0.89 (s, 9H), 0.06 (s, 6H).

**4-Fluoro-5-isopropyl-2-methoxy-3-(4-morpholinopiperidin-1-yl)-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (ALK-73).** 4-Morpholinopiperidine (0.5 g) and DIPEA (0.5 mL) were added to a solution of 3,4-difluoro-5-isopropyl-2-methoxy-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (135 mg, 0.37 mmol) in DMSO (2 mL) and the mixture was heated to 120 to 140 °C for 3 days. The reaction mixture was cooled down to RT and purified by preparative HPLC to afford the title compound (24 mg, 15% yield). $^1$H NMR (300 MHz, $CD_3OD$) $\delta$ ppm 8.21 (d, $J$ = 8.0 Hz, 1H), 7.64 (s, 1H), 7.60 (s, 1H), 7.39 (d, $J$ = 8.0 Hz, 1H), 5.10−4.90 (m, 1H), 4.20−4.10 (m, 2H), 4.00 (s, 3H), 3.90−3.70 (m, 2H), 3.65−3.50 (m, 4H), 3.45−3.20 (m, 5H), 2.33−2.20 (m, 2H), 2.10−1.85 (m, 2H), 1.70 (dd, $J$ = 6.9, 1.9 Hz, 6H).

**4-Fluoro-5-isopropyl-2-methoxy-3-(1-methylpiperidin-4-yl)-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (CJ-2212).** Methyl 6-cyano-2-((2-fluoro-4-methoxy-3-(1-methylpiperidin-4-yl)phenyl)(isopropyl)amino)-1*H*-indole-3-carboxylate (180 mg, 0.38 mmol) was dissolved in diphenyl ether (10 mL) and the solution was refluxed for 1 h. The mixture was cooled down to RT. Hexane (20 mL) was added and the precipitate was collected by filtration. The precipitate was purified by preparative HPLC to afford the title compound (100 mg, 59% yield). $^1$H NMR (300 MHz, $CD_3OD$) $\delta$ ppm 8.25 (d, $J$ = 8.0 Hz, 1H), 7.76 (s, 1H), 7.67 (s, 1H), 7.44 (d, $J$ = 8.0 Hz, 1H), 5.10−5.00 (m, 1H), 4.03 (s, 3H), 3.80−3.62 (m, 3H), 3.30−3.12 (m, 2H), 2.96 (s, 3H), 2.80−2.60 (m, 2H), 2.18−2.00 (m, 2H), 1.73 (dd, $J$ = 7.0, 2.0 Hz, 6H).

**4-Fluoro-5-isopropyl-3-(1-isopropylpiperidin-4-yl)-2-methoxy-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (CJ-2225).** Methyl 6-cyano-2-((2-fluoro-3-(1-isopropylpiperidin-4-yl)-4-methoxyphenyl)(isopropyl)amino)-1*H*-indole-3-carboxylate (150 mg, 0.296 mmol) was dissolved in diphenyl ether (10 mL) and the solution was refluxed for 1 h. The mixture was cooled down to RT. Hexane (20 mL) was added and the precipitate was collected by filtration. The precipitate was purified by preparative HPLC to afford the title compound (35 mg, 25% yield). $^1$H NMR (300 MHz, $CD_3OD$) $\delta$ ppm 8.26 (d, $J$ = 8.0 Hz, 1H), 7.76 (s, 1H), 7.68 (s, 1H), 7.44 (d, $J$ = 8.0 Hz, 1H), 5.10−5.00 (m, 1H), 4.03 (s, 3H), 3.80−3.50 (m, 4H), 3.30−3.20 (m, 2H), 2.80−2.60 (m, 2H), 2.15−2.03 (m, 2H), 1.73 (dd, $J$ = 7.0, 2.0 Hz, 6H), 1.45 (d, $J$ = 6.7 Hz, 6H).

**4-Fluoro-5-isopropyl-2-methoxy-11-oxo-3-(1-(tetrahydro-2*H*-pyran-4-yl)piperidin-4-yl)-6,11-dihydro-5*H*-indolo[2,3-*b*]-quinoline-8-carbonitrile (CJ-2226).** Methyl 6-cyano-2-((2-fluoro-4-methoxy-3-(1-(tetrahydro-2*H*-pyran-4-yl)piperidin-4-yl)phenyl)-(isopropyl)amino)-1*H*-indole-3-carboxylate (140 mg, 0.255 mmol) was dissolved in diphenyl ether (10 mL) and the solution was refluxed for 1 h. The mixture was cooled down to RT. Hexane (20 mL) was added and the precipitate was collected by filtration. The precipitate was purified by preparative HPLC to afford the title compound (70 mg, 53% yield). $^1$H NMR (300 MHz, $CD_3OD$) $\delta$ ppm 8.25 (d, $J$ = 8.0 Hz, 1H), 7.75 (s, 1H), 7.66 (s, 1H), 7.43 (d, $J$ = 8.0 Hz, 1H), 5.15−4.95 (m, 1H), 4.20−4.07 (m, 2H), 4.03 (s, 3H), 3.82−3.70 (m, 3H), 3.60−3.40 (m, 3H), 3.30−3.20 (m, 2H), 2.80−2.60 (m, 2H), 2.20−1.80 (m, 6H), 1.73 (dd, J = 6.9, 1.8 Hz, 6H).

**4-Fluoro-5-isopropyl-2-methoxy-3-(1-(oxetan-3-yl)-piperidin-4-yl)-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]-quinoline-8-carbonitrile (CJ-2224).** Methyl 6-cyano-2-((2-fluoro-4-methoxy-3-(1-(oxetan-3-yl)piperidin-4-yl)phenyl)(isopropyl)-amino)-1*H*-indole-3-carboxylate (190 mg, 0.37 mmol) was dissolved in diphenyl ether (10 mL) and the solution was refluxed for 1 h. The mixture was cooled down to RT. Hexane (20 mL) was added and the precipitate was collected by filtration. The precipitate was purified by preparative HPLC to afford the title compound (90 mg, 51% yield). $^1$H NMR (300 MHz, $CD_3OD$) $\delta$ ppm 8.28 (d, $J$ = 8.0 Hz, 1H), 7.78 (d, $J$ = 1.1 Hz, 1H), 7.69 (d, $J$ = 0.7 Hz, 1H), 7.46 (dd, $J$ = 8.0, 1.1 Hz, 1H), 5.18−5.02 (m, 1H), 4.95−4.80 (m, 4H), 4.55−4.40 (m, 1H), 4.04 (s, 3H), 3.80−3.60 (m, 3H), 3.20−3.10 (m, 2H), 2.80−2.60 (m, 2H), 2.18−2.02 (m, 2H), 1.74 (dd, $J$ = 7.0, 2.1 Hz, 6H).


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**4-Fluoro-3-(1-(2-hydroxyethyl)piperidin-4-yl)-5-isopropyl-2-methoxy-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-8-carbonitrile (CJ-2227).** Methyl 2-((3-(1-(2-((*tert*-butyldimethylsilyl)oxy)ethyl)piperidin-4-yl)-2-fluoro-4-methoxyphenyl)(isopropyl)amino)-6-cyano-1*H*-indole-3-carboxylate (180 mg, 0.29 mmol) was dissolved in diphenyl ether (10 mL) and the solution was refluxed for 1 h. The mixture was cooled down to RT. Hexane (20 mL) was added and the precipitate was collected by filtration. The precipitate was dissolved in MeOH (10 mL) and the solution was treated with $KHSO_4$ (197 mg, 1.45 mmol). The mixture was stirred at RT for 12 h. The solvent was removed under reduced pressure and the residue was purified by preparative HPLC to afford the title compound (80 mg, 58% yield). $^1$H NMR (300 MHz, $CD_3OD$) $\delta$ ppm 8.26 (d, $J$ = 8.0 Hz, 1H), 7.75 (s, 1H), 7.68 (s, 1H), 7.44 (d, $J$ = 8.0 Hz, 1H), 5.20−5.02 (m, 1H), 4.03 (s, 3H), 4.00−3.84 (m, 2H), 3.80−3.60 (m, 3H), 3.35−3.20 (m, 4H), 2.80−2.60 (m, 2H), 2.15−2.00 (m, 2H), 1.73 (dd, $J$ = 6.9, 1.6 Hz, 6H).

**Enzymatic Inhibition Assay.** The cytoplasmic domain (amino acid 1058−1620) of wild-type human ALK protein expressed as N-terminal GST-fusion protein was purchased from Carna Biosciences, Inc. (Japan). Mutated ALK proteins were expressed in SF9 insect cells with N-terminal tags cleaved after purification. Kinase activities of all enzymes were assessed using a Lance TR-FRET assay kit from PerkinElmer Life Sciences (Waltham, MA). Compound solution (2.5 $\mu$L) and 5 $\mu$L of protein solution were added into a black low volume 384-well microtiter plate, which was incubated for 30 min with gentle shaking at RT, followed by addition of 2.5 $\mu$L of fluorescently labeled peptide substrate (Ulight-CKKSRGDYMTMQIG) and ATP mixture solution. The kinase reaction was performed in 50 mM HEPES (pH 7.5) with 1 mM EGTA, 1 mM $MgCl_2$, and 2 mM DTT, and 0.01% Tween 20 was added immediately prior to the assay. Final concentrations of ATP, substrates, and DMSO were 100 $\mu$M, 20 nM, and 0.5%, respectively. Concentrations of different ALK proteins were adjusted accordingly to achieve comparable enzymatic activities for both wild-type and all mutated ALK proteins. The final ALK concentrations were 1, 1, 1, 128, 2, and 4 nM for wild type, F1174L, L1196M, S1206Y, G1269A, and G1202R, respectively. The reaction was allowed to proceed for 90 min in the dark with gentle shaking at RT after which 10 $\mu$L of 20 mM EDTA and 2 nM Eu-W1024 anti-phosphotyrosine antibody (PT66) mixture solution in the detection buffer from the manufacturer was added to terminate the reaction and detect the phosphorylation of the peptide substrate. The final mixture was incubated in the dark for 1 h before the plate was read on a Tecan Infinite $M^{-1000}$ multimode plate reader (Tecan, Durham, NC) with an excitation wavelength of 320 nm. Emission intensities were measured at both 620 and 665 nm with the intensity ratio between 665 and 620 nm corresponding to the peptide substrate phosphorylation. $IC_{50}$ values of inhibitors were obtained by fitting the ratio of 665/620 nm *vs* inhibitor concentrations in a sigmoidal dose−response curve (variable slope) with a nonlinear regression.

**Kinase Selectivity Enzymatic Assays.** The experiments were conducted at DiscoveRx Corporation. For most assays, kinase-tagged T7 phage strains were prepared in an *Escherichia coli* host derived from the BL21 strain. *E. coli* were grown to log phase, infected with T7 phage, and incubated with shaking at 32 °C until lysis. The lysates were centrifuged and filtered to remove cell debris. The remaining kinases were produced in HEK-293 cells and subsequently tagged with DNA for qPCR detection. Streptavidin-coated magnetic beads were treated with biotinylated small-molecule ligands for 30 min at RT to generate affinity resins for kinase assays. The liganded beads were blocked with excess biotin and washed with blocking buffer (SeaBlock (Pierce), 1% BSA, 0.05% Tween 20, and 1 mM DTT) to remove unbound ligand and to reduce nonspecific binding. Binding reactions were assembled by combining kinases, liganded affinity beads, and test compounds in 1× binding buffer (20% SeaBlock, 0.17× PBS, 0.05% Tween 20, and 6 mM DTT). All reactions were performed in polystyrene 96-well plates in a final volume of 0.135 mL. The assay plates were incubated at RT with shaking for 1 h and the affinity beads were washed with wash buffer (1× PBS and 0.05% Tween 20). The beads were then resuspended in elution buffer (1× PBS, 0.05% Tween 20, and 0.5 $\mu$M nonbiotinylated affinity ligand) and incubated at RT with shaking for 30 min. The kinase concentration in the eluates was measured by qPCR.

**Cell Viability Assay.** The effect of compounds on cell viability was determined in a 4 day proliferation assay. Cells were maintained in RPMI1640 culture medium with 10% FBS at 37 °C and an atmosphere of 5% $CO_2$. All the cell lines were used within 3 months of thawing fresh vials. Cells were seeded in 96-well plates at a density of 3000−10,000 cells/well in 75 $\mu$L of culture medium. Compounds were serially diluted in the appropriate medium, and 75 $\mu$L of the diluted compounds was added to the appropriate wells of the cell plate. After the addition of compounds, the cells were incubated at 37 °C in an atmosphere of 5% $CO_2$ for 4 days. Cell viability was determined using the CellTiter-Glo Luminescent Cell Viability Assay Kit (Promega, Madison, WI) (for KARPAS-299 cells) or WST (2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfo-phenyl)-2*H*-tetrazolium, monosodium salt) Cell Counting-8 Kit (Dojindo Molecular Technologies, Inc., Rockville, MD) (for H3122 cells) according to the manufacturer's instructions.

For WST assays, the WST-8 reagent was added to each well at a final concentration of 10% (v/v), and then the plates were incubated at 37 °C for 1−2 h for color development. The absorbance was measured at 450 nm using a SPECTRAmax PLUS plate reader (Molecular Devices, Sunnyvale, CA). The readings were normalized to the DMSO-treated cells and the half-maximal inhibitory concentration ($IC_{50}$) was calculated by nonlinear regression analysis using GraphPad Prism 5 software (GraphPad Software, La Jolla, CA).

For CellTiter-Glo assay, 100 $\mu$L of CellTiter-Glo Reagent was added to each well, and then the plates were incubated at RT for 10−20 min. The luminescent signal was measured using a Tecan Infinite M1000 multimode microplate reader (Tecan, Morrisville, NC). The readings were normalized to the DMSO-treated cells and the $IC_{50}$ values were calculated by nonlinear regression analysis using GraphPad Prism 5 software.

**Computational Docking Simulations.** All the docking simulations were performed using GOLD (version 4.0.11).[34,35] The ALK structural coordinates were extracted from the cocrystal structure of ALK in complex with CH5424802 (PDBID: 3AOX3)[27,35] and used for the docking calculations. ALK protein and the designed compounds were prepared using the MOE program.[36] The N- and C-termini of ALK were capped with a methyl group and acetyl group, respectively, and the missing hydrogen atoms were added by setting the pH at 7.0. The binding site of ALK was centered at Glu1132 with a radius of 20 Å. In each genetic algorithm (GA) run, a maximum number of 200,000 operations were set on a population of 5 islands of 100 individuals. Operator weights for crossover, mutation, and migration were set to 95, 95, and 10, respectively. Twenty docking runs were used for each compound. The PLP scoring function in the GOLD program[35] was used as the fitness function to assess the docked conformations. The highest ranked docked conformation of compounds **7d** and CJ-2360 were selected as their predicted binding poses, as shown in Figures 2 and 3, respectively.

***In Vivo* Pharmacodynamic and Efficacy Studies.** Human anaplastic large cell lymphoma (ALCL) KARPAS-299 cells were purchased from Sigma and maintained at 37 °C, 95% air, and 5% carbon dioxide in RPMI-1640 medium (with 2.05 mM L-glutamine; 0.1 $\mu$M, sterile filtered) supplemented with 10% fetal bovine serum, 100 units/mL penicillin, and 100 units/mL streptomycin (GIBCO, Invitrogen Corp.) and passaged twice weekly.

Tumor cells for xenografts were passaged into fresh medium the day before injection. For injection, a cell sample was mixed 1:1 with Trypan Blue (GIBCO, Invitrogen Corp.) and counted on a hemocytometer to determine the number of live/dead cells. Cells were washed twice with 1× PBS (GIBCO, Invitrogen Corp.) and resuspended in an ice-cold mixture of 1:1 PBS and Matrigel (BD Biosciences, Invitrogen Corp.) for a final Matrigel protein concentration of 5 mg/mL. All tumors were inoculated subcutaneously (s.c.) into female SCID mice (strain: 236 C.B-17 SCID, Charles River) with $5 \times 10^6$ KARPAS-299 cells in 0.1 mL with Matrigel in each mouse. The size of tumors growing in the mice was measured in two dimensions using calipers. Tumor volume ($mm^3$)


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= $(A \times B^2)/2$, where $A$ and $B$ are the tumor length and width (in mm), respectively.

For pharmacodynamic (PD) experiments, tumors were grown to 200−600 $mm^3$ and mice were randomly assigned for a single oral dose of CJ-2360 or alectinib. The control group mice were treated with vehicle alone. Tumor samples were collected at indicated time points post dose. Control mice were given vehicle only and collected at the 6 h time point. The mice were euthanized with $CO_2$ and 0.3−0.5 mL of blood was drawn from a cardiac puncture using a 25g needle and 1 mL syringe, which was pretreated with heparin. Tumors were removed and samples were placed in cassettes in 10% neutral-buffered formalin for 24 h and then transferred to 70% ethanol. Tumor sample was immediately frozen in liquid nitrogen, ground into fine powder, and stored at −80 °C.

For western blot analysis, tumor tissues were lysed with radioimmunoprecipitation assay buffer (RIPA buffer). The protein concentration of tissue lysate was determined with Bio-Rad Protein Assay Dye Reagent Concentrate (#5000006). The levels of phosphorylated STAT3 protein (pTyr705 rabbit, Cell Signaling Technology, Beverly, MA), phosphorylated AKT protein (pSer473, rabbit, Cell Signaling Technology, Beverly, MA), and phosphorylated ERK protein (pThr202/Tyr204, rabbit, Cell Signaling Technology, Beverly, MA) in tissue lysate were examined by western blotting analysis. GAPDH (sc-25,778, Santa Cruz Biotechnology) was used as a loading control.

For the efficacy study, the size of tumors growing in the mice was measured in two dimensions using calipers. Tumor volume ($mm^3$) = $(A \times B^2)/2$, where $A$ and $B$ are the tumor length and width (in mm), respectively. During treatment, tumor volume and body weight were measured three times a week. After the treatment was stopped, tumor volume and body weight were measured at least once a week. Before treatment began, tumors were allowed to grow to an average of 150 $mm^3$ (70−270 $mm^3$) in volume. Mice with tumors within the acceptable size range were randomized into treatment groups of seven to eight mice for each group. CJ-2360 or alectinib was administered *via* oral gavage, twice or once daily for 3 weeks. Mice in the control group received vehicle alone once daily for 3 weeks.

All animal experiments were performed under the guidelines of the University of Michigan Committee for Use and Care of Animals and using an approved animal protocol (PI, Shaomeng Wang).

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jmedchem.0c01550.

Summary of KINOMEscan screening data for CJ-2360 against 468 kinases; HPLC trace of CJ-2360 (PDF)

Modeled structure for compound **7d** in complex with ALK (PDB)

Modeled structure for CJ-2369 in complex with ALK (PDB)

Molecular string file for all the final target compounds (CSV)

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¶J.C., Y.Z., and X.D. contributed equally to this work.



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### Notes

The authors declare the following competing financial interest(s): Ascentage has filed a patent application on this



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class of ALK inhibitors, in which S. Wang, J. Chen, Y. Zhou L. Jiao, Y. Jing, X. Qian, L. Liu, L. Bai, C-Y. Yang, and D. McEachern are co-inventors. S. Wang is a paid consultant of Ascentage and owns stock in Ascentage. J. Chen, Y. Zhou L. Jiao, Y. Jing, X. Qian, L. are employees of Ascentage and owns stock in Ascentage. The University of Michigan also owns stock in Ascentage.

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