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Synthesis and biological evaluation of benzo[4,5]imidazo[1,2-*c*]pyrimidine and benzo[4,5]imidazo[1,2-*a*]pyrazine derivatives as anaplastic lymphoma kinase inhibitors

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ABSTRACT

Chromosomal translocations involving anaplastic lymphoma kinase (ALK) are the driving mutations for a range of cancers and ALK is thus considered an attractive therapeutic target. We synthesized a series of functionalized benzo[4,5]imidazo[1,2-*c*]pyrimidines and benzo[4,5]imidazo[1,2-*a*]pyrazines by an aza-Graebe–Ullman reaction, followed by palladium-catalyzed cross-coupling reactions. A sequential regioselective cross-coupling route is reported for the synthesis of unsymmetrically disubstituted benzo[4,5]imidazo[1,2-*a*]pyrazines. The inhibition of ALK was evaluated and compound **19** in particular showed good activity against both the wild type and crizotinib-resistant L1196M mutant in vitro and in ALK-transfected BaF3 cells.

1. Introduction

Anaplastic lymphoma kinase (ALK) is a receptor tyrosine kinase which belongs to the insulin receptor family, initially identified as the catalytic domain of the NPM/ALK fusion kinase, expressed in most CD30+ anaplastic large-cell lymphoma (ALCL).[1] Since then, a variety of oncogenic fusion proteins resulting from chromosomal translocations of the intracellular domain of ALK have been identified in a wide range of tumors, including non-small cell lung cancer (NSCLC), inflammatory myofibroplastic tumor, diffuse large B-cell lymphoma, breast cancer, colon cancer, and others.[2,3] While the amino terminal partner of the fusion varies from case to case, it always provides a strong homodimerization interface, thus causing aberrant ligand-independent activation of ALK kinase activity. In addition, kinase-activating point mutations of full-length ALK have been reported and biologically validated in hereditary and sporadic neuroblastomas as well as in anaplastic thyroid carcinomas.[4,5] The oncogenic role of ALK has been defined in numerous preclinical models.[6] Therefore, the selective inhibition of ALK emerged as an attractive target for cancer therapies.[7] Indeed, the first clinical inhibitor of ALK, crizotinib, yielded impressive responses in patients with advanced NSCLC and ALCL, although acquired resistance invariably develops, often as a result of point mutations within the ALK catalytic domain.[8,9] A large effort is ongoing to develop more potent second-generation inhibitors that are able to overcome such drug resistance.[3]

Chugai and Roche's tetracyclic clinical candidate AF802 as well as our own results with substituted α-carbolines have shown that fused nitrogen-containing heterocyclic systems are privileged skeletons for targeting the ALK tyrosine kinase domain (Fig. 1).[10] We therefore turned our attention to the development of selective

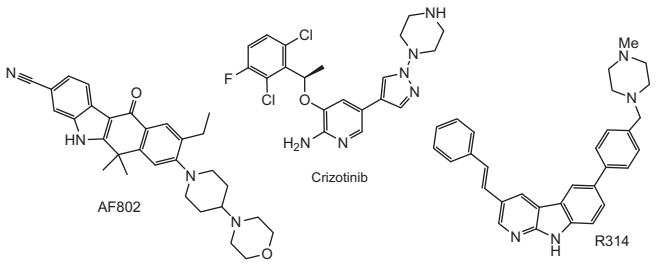

Figure 1. Alk inhibitors.

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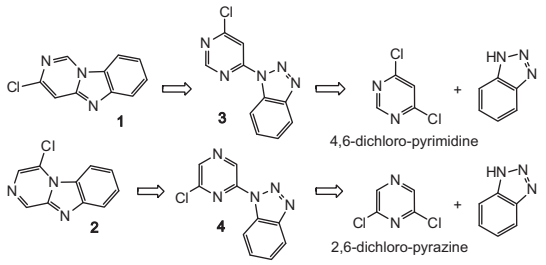


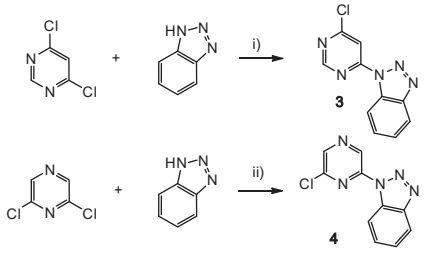
Scheme 1. Synthetic route to target heterocyclic cores.

inhibitors of ALK based on two original heterocyclic cores: the benzo[4,5]imidazo[1,2-*c*]pyrimidine **1** and the benzo[4,5]imidazo[1,2-*a*]pyrazine **2**. These two tricyclic systems were synthesized by a route analogous to that reported by Bergman and Vallberg,[11] with an added halogen substituent in order to incorporate diversity at a late stage through transition metal catalyzed chemistry (Scheme 1). Condensation of either 4,6-dichloropyrimidine or 2,6-dichloropyrazine with benzotriazole affords compounds **3** and **4**, which undergo a Graebe–Ullman-like cyclization to produce **1** and **2**, respectively.

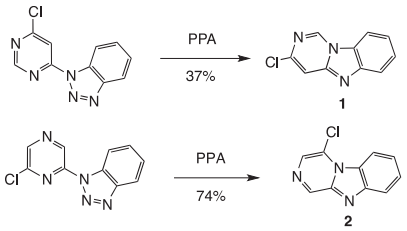
2. Results and discussion

Optimization of the coupling of the dichlorodiazines with benzotriazole was necessary, as both systems contain two equivalent electrophilic sites (Scheme 2). Indeed, in the case of the more reactive 4,6-dichloropyrimidine, heating a neat equimolar ratio of the two reagents led predominantly to the double substitution product. It was therefore necessary to work with 2 equiv of 4, 6-dichloropyrimidine in order to favor the formation of the mono-substitution product **3**. Compound **3** was thus obtained in 87% yield after 2 h by starting the reaction at 80 °C and then increasing the temperature to 110 °C.

The reactivity of 2,6-dichloropyrazine, in contrast, was rather poor. As the dichloropyrazine tended to sublimate at temperatures below those required for the reaction, it was necessary to work in a sealed tube at 155 °C for 24 h to provide the desired intermediate **4** in a respectable 60% yield (Scheme 2).



Scheme 2. Reagents and conditions: (i) 4,6-dichloropyrimidine (2 equiv), benzotriazole (1 equiv), neat, 80–110 °C, 2 h, **3** = 87%. (ii) 2,6-Dichloropyrazine (1 equiv), benzotriazole (1.1 equiv), neat, sealed tube, 155 °C, 24 h, **4** = 60%.



Scheme 3. Synthesis of heterocyclic skeletons **1** and **2**.

Polyphosphoric acid (PPA)-mediated cyclization of intermediates **3** and **4** produced the desired compounds **1** and **2**, respectively, in satisfactory yields (Scheme 3). It should be noted that the N-cyclized products **1** and **2** were obtained exclusively, rather than the C-cyclized diazacarbazoles (9*H*-indolo[2,3-*e*]pyrimidine and 9*H*-indolo[2,3-*b*]pyrazine). This selectivity is opposite that which we observed with 1-pyrid-2-ylbenzotriazoles, which gave exclusively the C-cyclized 9*H*-indolo[2,3-*b*]pyridines.[10a] Moreau et al. observed a mixture of the N-cyclized 1*H*-Pyrrolo[3′,2′:5,6]pyrido[1,2-*a*]benzimidazole and C-cyclized 1,9-dihydropyrrolo[3′,2′:5,6]pyrido[2,3-*b*]indole in the cyclization of 7-azaindol-6-ylbenzotriazoles at high temperatures.[12a] A number of C-cyclization and N-cyclization reactions have been reported in the literature and the selectivity appears to depend on steric and electronic factors as well as experimental conditions, although the basis for the selectivity remains unclear.[12]

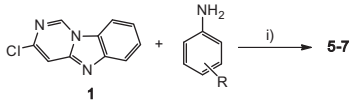
We then investigated the functionalization of these two heterocyclic structures using palladium-catalyzed coupling reactions. Compound **1**, for example, bearing a chlorine atom at the α position to a nitrogen, was expected to be highly reactive under Pd-catalyzed coupling conditions. Surprisingly, however, no Suzuki–Miyaura reaction[13] was observed using either $Pd(Ph_3)_4$ or $Pd_2(dba)_3$/X-Phos in the coupling reaction with boronic acid partners. The starting material was recovered intact.

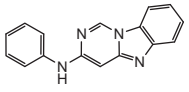
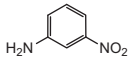
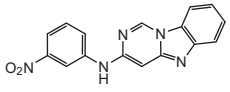
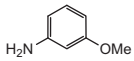
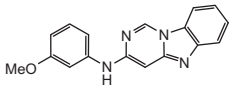
In contrast, the Buchwald–Hartwig cross coupling reaction[14] worked well using $Pd_2(dba)_3$/X-Phos in combination with aromatic amines. Therefore, the reaction was performed with aniline and two different substituted anilines to provide derivatives **5**, **6** and **7** in good yields (Table 1). The difference in outcome between the two reactions suggests that the transmetallation or reductive elimination steps were at the origin of the lack of reactivity in the Suzuki–Miyaura reaction, rather than the oxidative insertion.

In order to develop a sequential cross-coupling strategy, we decided to investigate the regiochemistry of the bromination of compound **1**. Reaction of 3-chlorobenzo[4,5]imidazo[1,2-*c*]pyrimidine (**1**) with *N*-bromosuccinimide (NBS) gave the dihalogenated 8-bromo derivative **8** in 68% yield (Scheme 4). The structure of compound **8** was confirmed by NOE experiments.

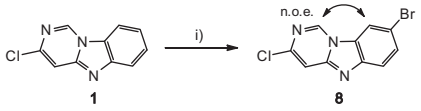
The Buchwald–Hartwig coupling reaction between compound **8** and aniline, using $Pd_2(dba)_3$/X-Phos, gave compound **9** in disappointing 16% yield. We therefore investigated the Suzuki–Miyaura coupling with $Pd(Ph_3)_4$ and 4-methoxyphenyl boronic acid. Once again, the coupled product **10** was isolated in low yield (9%) (Scheme 5). In these two reactions, the starting material was not

Table 1
Buchwald reactions on 3-chloro-benzo[4,5]imidazo[1,2-*c*]pyrimidine (**1**)



Entry	Anilines	Products		Yield (%)
1			**5**	67
2			**6**	95
3			**7**	65

Reaction conditions: (i) $Pd_2(dba)_3$ (8 mol %), X-Phos (16 mol %), K_2CO_3 (3 equiv), *t*-BuOH (*C* = 0.2 M), anilines (1.5 equiv), 100 °C, overnight.



Scheme 4. Reagents and comditions: (i) *N*-bromo-succinimide (1.5 equiv), THF (C = 0.1 M), rt, 15 h, **8** = 68%.

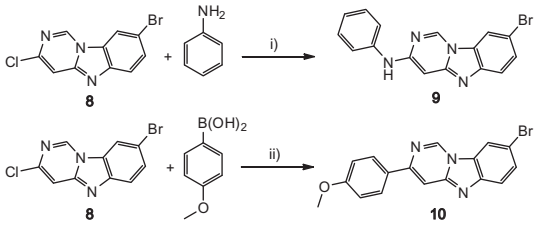
recovered and the remaining material consisted of a complex mixture of degradation products, which were not characterized. Nevertheless, surprisingly, the reaction was completely regioselective in favor of the chlorine-substituted position, as neither the 8-substituted nor the disubstituted compound was detected.

Given the disappointing reactivity of compounds **1** and **8** in cross coupling reactions, we turned our attention to the study of the reactivity of core structure **2**. We investigated the bromination of compound **2** with bromine or *N*-bromo-succinimide to provide **11** (Scheme 6). Using bromine, the desired dihalogenated compound was obtained in 53% yield, but compound **11** was not easily separated from the remaining starting material. On the other hand, bromination with NBS was slower and less efficient (only 29% yield) but the purification was easier due to the absence of starting material. The position of the bromine atom on the heterocyclic structure was confirmed by ^{15}N–^{1}H NMR HMBC correlation.

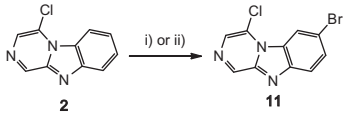
When the dihalogenated benzo[4,5]imidazo[1,2-*a*]pyrazine **11** was submitted to Suzuki–Miyaura coupling with $Pd(Ph_3)_4$ at 70 °C using a slight excess of boronic acid or boronic acid pinacol ester a lack of regioselectivity was observed. Indeed, using the boronic acid, the conversion was satisfactory, but we obtained a mixture of both mono- and disubstituted products. The corresponding reaction using the boronic acid pinacol ester at 70 °C (Scheme 7) provided an inseparable 7:3 mixture of the two mono-substituted compounds in 33% yield in which the major compound was substituted on the chlorinated position.

Moreover, we obtained complete conversion of the starting material to the disubstituted structure using an excess (3 equiv) of the boronic acid pinacol ester and working at 100 °C overnight. Compound **12** was obtained in a high 77% yield by this procedure (Scheme 8).

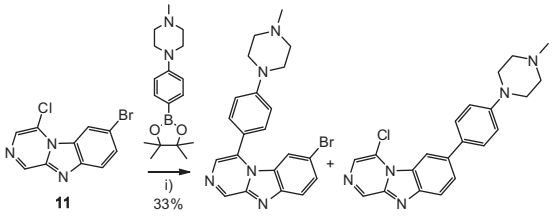
In order to increase the regioselectivity and build an efficient sequential route for functionalization of the heterocyclic core **11**, we replaced the bromine atom by chlorine. Thus, pyrazino-benzimidazol **2** was chlorinated in the same position by *N*-chloro-succinimide to give the dichlorinated compound **13** in a moderate 54% yield (Scheme 9).



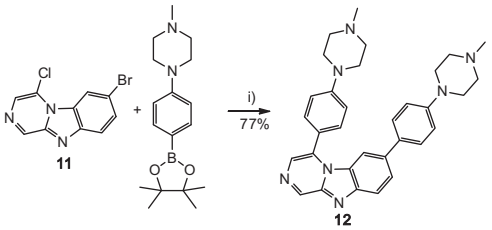
Scheme 5. Reagents and conditions: (i) $Pd_2(dba)_3$ (8 mol %), X-Phos (16 mol %), K_2CO_3 (3 equiv), *t*-BuOH (C = 0.2 M), aniline (1.5 equiv), 100 °C, overnight, **9** = 16%. (ii) $Pd(Ph_3)_4$ (10 mol %), K_2CO_3 (3 equiv), dioxane/H_2O 5:1, boronic acid (1.2 equiv), 70 °C, overnight, **10** = 9%.



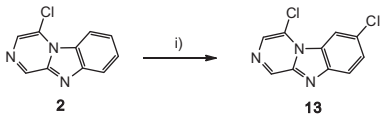
Scheme 6. Reagents and conditions: (i) Br_2 (2 equiv), DCM (C = 0.2 M), rt, 16 h, **11** = 53%. (ii) NBS (2.5 equiv), DMF (C = 0.1 M), rt, 24 h, **11** = 29%.



Scheme 7. Reagents and conditions: (i) $Pd(Ph_3)_4$ (10 mol %), K_2CO_3 (3 equiv), ester pinacol borane (1.3 equiv), dioxane/H_2O 5:1, 70 °C, overnight.



Scheme 8. Reagents and conditions: (i) $Pd(Ph_3)_4$ (10 mol %), K_2CO_3 (4 equiv), ester pinacol borane (3 equiv), dioxane/H_2O 5:1, 100 °C, overnight.



Scheme 9. Reagents and conditions: (i) NCS (1.6 equiv), DMF (C = 0.1 M), rt, 21 h, **13** = 54%.

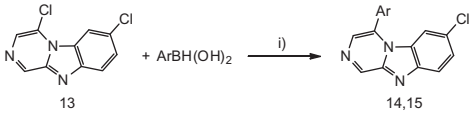
As expected, when compound **13** was submitted to Suzuki–Miyaura coupling reaction, the selectivity was strongly in favor of position 4. The reaction occurred only on the activated chlorinated centre, leaving the chlorine on the phenyl moiety unreacted. Using *p*-methoxyphenyl boronic acid and vinylphenyl boronic acid, we synthesized compounds **14** and **15** in good yields (Table 2). As expected, the best result was obtained using the more electron rich 4-methoxyphenyl boronic acid.

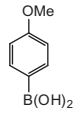
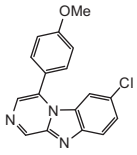
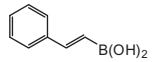
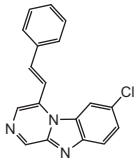
We then turned our attention to functionalizing the other chlorinated position of **14** and **15**. It was necessary to use more drastic conditions using the $Pd(OAc)_2$/S-Phos system, 2 equiv of the coupling partner, at 100 °C in *n*-butanol for 16 h. Starting from compound **14**, three products **16**, **17**, and **18** were prepared in moderate to good yields (Table 3). In order to use boronic acid derivatives with a different range of reactivity, size and polarity, we selected three different boronic reagents: 4-*N*-acetamido-phenyl boronic acid, 4-(4-*N*-methyl-piperazin-1-yl)phenyl boronic acid pinacol ester and *E*-styryl boronic acid.

Finally, in order to illustrate the scope of our strategy, we synthesized the inverse-substituted analogue of compound **18** from **15**, using 4-methoxyphenyl boronic acid under the same conditions (Scheme 10). Thus, we obtained the derivative **19** in a fair 41% yield. These results demonstrate that the sequential functionalization of the heterocyclic scaffold **2** was achieved, providing an original strategy for the orthogonal substitution of compound **13**.

The biological activities of compounds were assessed in a series of biological assays against ALK. The compounds were tested in an in vitro ELISA assay[15] using a more physiologically relevant concentration of ATP (300 μM), against both wild-type (wt) ALK catalytic domain and the crizotinib-resistant gatekeeper mutant

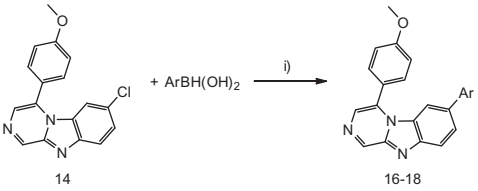
Table 2
Suzuki–Miyaura reactions on 4,7-dichloro-benzo[4,5]imidazo[1,2-*a*]pyrazine (**13**)

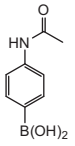
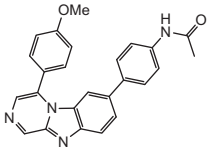
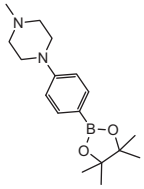
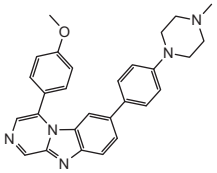
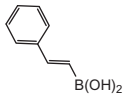
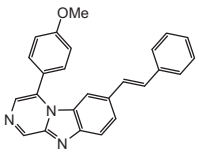


Entry	Boronic acid	Products		Yield (%)
1			**14**	86
2			**15**	67

(i) $Pd(Ph_3)_4$ (10 mol %), K_2CO_3 (3 equiv), boronic acid (1.3 equiv), THF/H_2O 5:1, 70 °C, overnight.

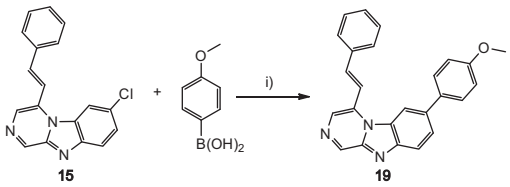
Table 3
Suzuki coupling reactions of 7-chloro-4-(4-methoxy-phenyl)-benzo[4,5]imidazo[1,2-*a*]pyrazine (**14**) under Buchwald conditions



Entry	Boronic acid	Products		Yield (%)
1			**16**	80
2			**17**	40
3			**18**	52

(i) $Pd(AcO)_2$ (10 mol %), S-Phos (20 mol %), K_3PO_4 (3 equiv), *n*-BuOH (C = 0.05 M), boronic acid or pinacol ester (2 equiv), 100 °C, 14 h.

L1196M. While ATP concentrations close to the K_m, near 10 μM, are typically used in order to maximize the number of inhibitors detected, we chose a more stringent assay, closer to the physiological concentration of 700 μM. In order to assess both cellular activity and specificity, selected compounds were tested against both

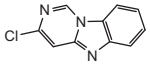
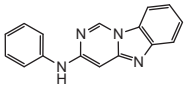
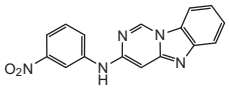
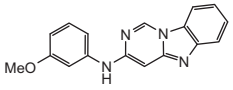
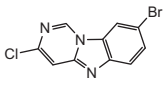
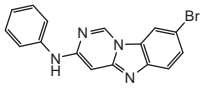
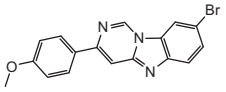


Scheme 10. Reagents and conditions: (i) $Pd(AcO)_2$ (10 mol %), S-Phos (20 mol %), K_3PO_4 (3 equiv), *n*-BuOH (C = 0.05 M), boronic acid or pinacol ester (2 equiv), 100 °C, 14 h, (**19**) = 41%.

parental Ba/F3 cells (control) and Ba/F3 cells transfected with either native NMP/ALK or its L1196M mutant. Criztoinib (Xalkori®) was used as a reference compound. It should be noted that the higher IC_{50} observed in our ELISA assay (1.6 μM) is by no means inconsistent with literature reports for an ATP-competitive inhibitor, as the [ATP] in our assay is considerably higher (see Supplementary material).

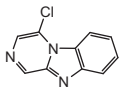
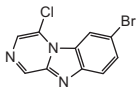
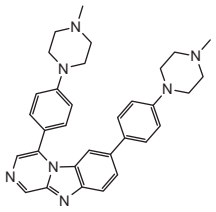
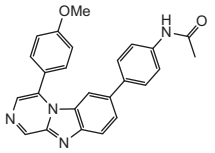
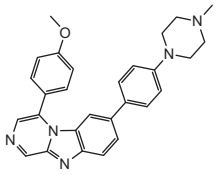
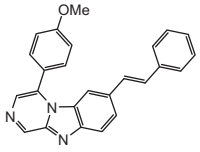
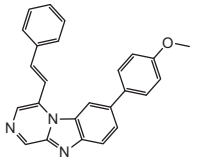
Neither of the two mono-chlorinated core compounds **1** and **2** themselves showed activity against ALK. Several of the imidazolo[1,2-*c*]pyrimidine compounds showed inhibition of the recombinant ALK kinase domain in the 10 micromolar range in vitro, such as the substituted compounds **6** and **10** (Table 4). The imidazolo[1,2-*a*]pyrazine derivatives, for example, **16**–**19**, gave more promising results (Table 5). Significantly, while crizotinib was approximately 10-fold less active against the L1196M gatekeeper mutant compared to wt enzyme, the compounds from both series were not significantly affected by this mutation. One derivative, 4-(2-phenylvinyl)-7-methoxyphenylbenzo[4,5]imidazolo[1,2-*a*]pyrazine (**19**), showed submicromolar activity in vitro against wt ALK, with comparable inhibition of the L1196M mutant, thus giving superior results to crizotinib in our assay. Importantly, compound **19** inhibited Ba/F3-NPM/ALK cell proliferation with over 1-log selectivity versus parental isogenic IL-3-dependent Ba/F3 cells (Ta-

Table 4
In vitro inhibition of wild type and crizotinib-resistant ALK by benzo[4,5]imidazo[1,2-*c*]pyrimidine derivatives

Entry	Compound		IC_{50} (μM) ALKwt	IC_{50} (μM) ALKL1196M
1		Crizotinib	1.6	10
2	**1**		>100	n.t.
3	**5**		52	10
4	**6**		10	14
5	**7**		51	n.t.
6	**8**		>50	n.t.
7	**9**		69	49
8	**10**		10	n.t.

n.t.-not tested.

Table 5
In vitro inhibition of wild type and crizotinib-resistant ALK by benzo[4,5]imidazo[1,2-*a*]pyrazine derivatives

Entry	Compound		IC_{50} (μM) ALKwt	IC_{50} (μM) ALKL1196M
1		Crizotinib	1.6	10
2	**2**		>100	n.t.
3	**11**		>50	n.t.
4	**12**		>100	29
5	**16**		18	n.t.
6	**17**		14	n.t.
7	**18**		3.5	4.5
10	**19**		0.65	1.5

n.t.-not tested.

ble 6), indicating selective cellular inhibition of NPM/ALK and lower general cytotoxicity than Crizotinib. The activity against the parent Ba/F3 cells is used as a test of selectivity, which may be more representative than in vitro screens, and a selectivity factor (SF) can be calculated by comparing the activity on the NMP/ALK-dependent cells to the parent cells.

Modeling studies were performed to assess the binding mode of the compounds to the ALK catalytic domain. The docking protocol was first validated by docking NVP-TAE684 into the ALK kinase catalytic domain (PDB 2XB7).[16] The inhibitor docked with a score of −41.69 (Table 1) reproducing the resolved crystallographic pose with a low RMSD. Compounds **8**, **9**, **10**, **11**, **18** and **19** were then docked into the same binding pocket. All derivatives established one hydrogen bond with the NH of hinge Met 1199 providing the scores listed in Table 7. The highest score of −31.3 was obtained with **19** (Table 7, Fig. 2) in agreement with the experimental data showing that this compound gives the best IC_{50} of 0.65 μM within the series investigated. The vinylphenyl substituent is positioned parallel to the gatekeeper L1196, which contributes to the favorable binding of this compound. Comparing the IC_{50} values of the compounds in our assay to the corresponding docking scores (Table 7) the derivatives screened can be clustered into four groups: Group 1 (NVP-TAE684, score −41.69, IC_{50} <0.1 μM), Group 2 (**19**, score −31.3, IC_{50} <1 μM), Group 3 (**18**, score −29.54, IC_{50} <10 μM), Group 4 (**8**, **10** and **11**, score <−29, IC_{50} >10 μM). The only outlier is compound **9** which should be part of Group 3 according to the score but possesses an IC_{50} above 50 μM. This can be explained by the overestimation of the contribution of the energy interaction of the aromatic secondary amine group of **9** in the docking pose obtained. More derivatives would be needed in order

Table 7
ICM docking scores

Entry	Compound	IC_{50} (μM) wt-ALK	Docking score
1	**8**	>50	−25.48
2	**9**	>50	−29.54
4	**10**	10	−25.97
5	**11**	>50	−26.23
6	**18**	3.5	−29.28
7	**19**	0.65	−31.30
8	TAE	0.056	−41.69

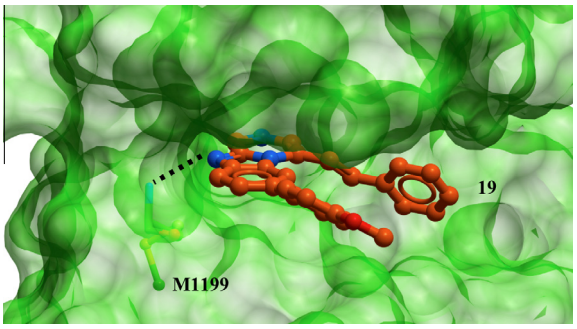


Figure 2. Docking pose of **19** in the human wild type-ALK kinase catalytic domain. The ligand is displayed as xstick and colored by atom type (carbon atoms in orange) and residue M1199 is displayed as xstick and colored by atom type (carbon atoms in green). The protein is displayed as Connolly Surface in green (ICM v. v3.7-3d). H-bonds are represented by black dashed lines between the donor (D) and the acceptor (A).

Table 6
Cell-based assays for ALK activity, as IC_{50} (μM)

Entry	Compound	ALKwt	ALKL1196M	BaF3 parent	BaF3 NPM/ALK	BaF3 NPM/ALKL1196M	SF[a]
1	Crizotinib	1.6	10	0.98	0.051	0.26	6
2	**9**	69	49	5.2	5.6	n.t.	1
3	**10**	10	n.t.	>25	17	9	>2
4	**11**	>50	n.t.	2.4	1.1	2.2	1
5	**12**	>100	29	0.87	0.34	0.20	3
6	**19**	0.65	1.5	>12.5	0.84	0.78	>15

[a] SF: selectivity factor = BaF3 parent/avg (BaF3 NPM/ALK, BaF3 NPM/ALK L1196M).

to come up with a reliable SAR, yet the model can provide some initial ideas for further elaboration of **19**.

In conclusion, we have developed an original route to 3-substituted benzo[4,5]imidazo[1,2-*c*]pyrimidines and 4,7-disubstituted benzo[4,5]imidazo[1,2-*a*]pyrazines. The parent monochlorinated compounds were prepared by an aza-Graebe–Ullman reaction, which after halogenation gave regioselectively dihalogenated analogs. These heterocyclic cores were functionalized through palladium-catalyzed coupling reactions to provide the mono- or disubstituted products. We further developed a sequential regioselective cross-coupling reaction using 4,7-dichlorobenzo[4,5]imidazo[1,2-*a*]pyrazine, which is powerful method for the preparation of large panels of unsymmetrically disubstituted compounds. These compounds are inhibitors of ALK with, in the case of compound **19**, better in vitro activity in our assay than crizotinib against both wt ALK and the crizotinib-resistant L1196M mutant. This compound also shows excellent selectivity for NPM/ALK and NPM/L1196M-ALK transfected Ba/F3 cells. Molecular docking shows that the compounds are able to bind into the ATP binding site of ALK forming a characteristic hydrogen bond with the hinge region, typical of protein kinase inhibitors.[17] These compound may serve as useful scaffolds for the development of selective inhibitors for ALK and other protein kinases.

3. Experimental

3.1. Synthesis

General experimental procedures are provided in the Supplementary material.

3.1.1. 1-(6-Chloropyrimidin-4-yl)-1*H*-benzo[*d*][1,2,3]triazole (3)

4,6-Dichloropyrimidine (2.32 g, 15.6 mmol, 2 equiv) and benzotriazole (0.928 g, 7.78 mmol) were placed in a 25 mL round-bottom flask attached to an open Vigreux condenser. The mixture was placed directly in an oil bath at 80 °C and then warmed to 110 °C. After stirring 2 h at 110 °C, the reaction mixture was cooled to room temperature and cautiously quenched with 25% aqueous ammonia. Then, 15 mL of DCM were added to the aqueous layer and stirred for 15 min. The organic layer was collected and the aqueous layer was extracted with DCM (60, 30 and 5 × 20 mL). The combined organic layers were dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure. A white powder was obtained in 87% yield (1.58 g) after purification by silica gel flash chromatography (PE/DCM: 50:50–30:70). $C_{10}H_6ClN_5$, 231.64 g mol^{-1}, ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.98 (d, J = 0.9 Hz, 1H), 8.59 (d, J = 8.3 Hz, 1H), 8.28 (d, J = 1.0 Hz, 1H), 8.12 (d, J = 8.2 Hz, 1H), 7.65 (ddd, J = 8.3, 7.1, 1.0 Hz, 1H), 7.49 (ddd, J = 8.2, 7.1, 1.0 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.8 (C), 158.9 (CH), 158.3 (C), 147.0 (C), 131.2 (C), 130.1 (CH), 126.0 (CH), 120.5 (CH), 115.1 (CH), 110.2 (CH); MS (ESI) m/z 232.0 $[M+H]^+$, 254.0 $[M+Na]^+$.

3.1.2. 1-(6-Chloropyrazin-2-yl)-1*H*-benzo[*d*][1,2,3]triazole (4)

In a sealed tube of 5 mL, 2,6-dichloropyrazine (359 mg, 2.41 mmol) and benzotriazole (316 mg, 2.65 mmol, 1.1 equiv) were heated at 155 °C for 24 h. After cooling to room temperature, the reaction mixture was cautiously quenched with 25% aqueous ammonia and diluted with DCM. After 15 min stirring, the organic layer was collected and the aqueous layer was extracted with DCM (3 × 20 mL). The combined organic layers were washed with a brine/water 1:1 mixture (20 mL), dried over $MgSO_4$, filtered and the solvent was removed in vacuo. A white powder was obtained in 60% yield (335 mg) after purification by silica gel flash chromatography (PE/DCM: 50:50–30:70). $C_{10}H_6ClN_5$, 231.64 g mol^{-1}, ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.53 (s, 1H), 8.59 (s, 1H), 8.47 (d, J = 8.4 Hz, 1H), 8.14 (d, J = 8.3 Hz, 1H), 7.65 (ft, J = 7.7 Hz, 1H), 7.50 (ft, J = 7.7 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 146.9 (C), 146.7 (2C), 141.6 (CH), 134.0 (CH), 131.3 (C), 129.9 (CH), 125.9 (CH), 120.4 (CH), 114.3 (CH); MS (ESI) m/z 232.0 $[M+H]^+$, 254.0 $[M+Na]^+$.

3.1.3. 3-Chlorobenzo[4,5]imidazo[1,2-*c*]pyrimidine (1)

In a 100 mL round-bottom flask, a mixture of the pyrimidinylbenzotriazole derivative **3** (297 mg, 1.28 mmol) and polyphosphoric acid (sufficient volume to cover the white solid) was heated from room temperature to 150 °C until the production of nitrogen ceased (2 h). The reaction mixture was then cooled to room temperature and suspended by trituration with a saturated solution of Na_2CO_3. The aqueous layer was slowly neutralized with a hydrochloride solution to pH 7 and extracted with DCM (2 × 60 mL) and AcOEt (2 × 60 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered and solvents were removed in vacuo. The crude product was triturated in MeOH and filtered. The filtrate was purified by silica gel flash chromatography (DCM/AcOEt: 100:0–90:10) to afford the desired compound, which combined to the solid resulting from the trituration, gave 37% yield (97 mg) as a light orange solid. $C_{10}H_6ClN_3$, 203.63 g mol^{-1}, ^{1}H NMR (400 MHz, DMSO) δ 9.91 (d, J = 1.0 Hz, 1H), 8.42 (d, J = 8.2 Hz, 1H), 7.84–7.86 (m, 2H), 7.61 (t, J = 7.7 Hz, 1H), 7.50 (t, J = 7.7 Hz, 1H); ^{13}C NMR (101 MHz, DMSO) δ 147.2 (C), 146.0 (C), 144.7 (C), 142.2 (CH), 127.1 (CH), 126.7 (C), 122.5 (CH), 119.3 (CH), 112.9 (CH), 110.0 (CH); MS (ESI) m/z 204.2 $[M+H]^+$.

3.1.4. 4-Chloro-benzo[4,5]imidazo[1,2-*a*]pyrazine (2)

In a 100 mL round-bottom flask, a mixture of the pyrazinylbenzotriazole derivative **4** (560 mg, 2.42 mmol) and polyphosphoric acid (sufficient volume to cover the white solid) was heated from room temperature to 150 °C until the production of nitrogen ceased (2 h). The reaction mixture was then cooled to room temperature and suspended by trituration with a saturated solution of Na_2CO_3. The aqueous layer was extracted with DCM (50 mL, 4 × 25 mL). The combined organic layers were washed with a brine/water 1:1 mixture (2 × 35 mL), dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure. A yellowish solid was obtained in 74% yield (364 mg) after purification by silica gel flash chromatography (PE/AcOEt: 70:30–60:40). $C_{10}H_6ClN_3$, 203.63 g mol^{-1}, ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.19 (s, 1H), 8.58 (d, J = 8.7 Hz, 1H), 8.07 (d, J = 8.4 Hz, 1H), 7.94 (s, 1H), 7.67 (ft, J = 7.8 Hz, 1H), 7.51 (ft, J = 7.9 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 144.5 (C), 143.5 (C), 143.1 (CH), 128.8 (C), 127.5 (CH), 126.6 (CH), 126.0 (C), 123.8 (CH), 121.5 (CH), 115.7 (CH); MS (ESI) m/z 204.2 $[M+H]^+$.

3.1.5. *N*-Phenyl-benzo[4,5]imidazo[1,2-*c*]pyrimidin-3-ylamine (5)

In a Schlenk tube with a stirring bar, 3-chloro-benzo[4,5]imidazo[1,2-*c*]pyrimidine **1** (40 mg, 0.196 mmol), K_2CO_3 (81 mg, 3 equiv), 2-dicyclohexylphosphino-2′,4′,6′-triisopropylbiphenyl (X-phos, 15 mg, 0.16 equiv), and $Pd_2(dba)_3$ (14 mg, 0.08 equiv) were placed. The tube was evacuated and back-filled with argon (repeated three additional times). Then, 1 mL of *t*-BuOH and aniline (27 μL, 1.5 equiv) were introduced (degassed solvents were used). The reaction mixture was allowed to stir at 100 °C for 15 h. After cooling to room temperature and quenching with water, the aqueous layer was extracted with DCM (2 × 15 mL) and AcOEt (2 × 15 mL). The combined organic extracts were washed with brine, dried over $MgSO_4$, filtered and the solvent was removed in vacuo. The crude product was purified by silica gel flash chromatography (PE/AcOEt 50:50–30:70) to provide the desired compound (34 mg) in 67% yield as a pale yellow solid. $C_{16}H_{12}N_4$, 260.29 g mol^{-1}, ^{1}H NMR (400 MHz,

$CDCl_3$) δ 8.98 (s, 1H), 7.81 (d, J = 8.0 Hz, 1H), 7.74 (d, J = 8.1 Hz, 1H), 7.47 (t, J = 7.7 Hz, 1H), 7.39 (t, J = 7.7 Hz, 2H), 7.35–7.27 (m, 3H), 7.15 (t, J = 7.2 Hz, 1H), 7.01 (s, NH), 6.85 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 152.8 (C), 150.8 (C), 146.1 (C), 139.0 (CH), 138.9 (C), 129.7 (CH), 127.0 (C), 126.6 (CH), 124.6 (CH), 121.6 (CH), 120.8 (CH), 119.0 (CH), 109.8 (CH), 85.2 (CH); HRMS calcd for $C_{16}H_{13}N_4$ $[M+H]^+$ 261.1135 found 261.1137.

3.1.6. *N*-(3-Nitrophenyl)-benzo[4,5]imidazo[1,2-*c*]pyrimidin-3-ylamine (6)

In a Schlenk tube with a stirring bar, 3-chlorobenzo[4,5]imidazo[1,2-*c*]pyrimidine **1** (40 mg, 0.196 mmol), K_2CO_3 (81 mg, 3 equiv), 2-dicyclohexylphosphino-2′,4′,6′-triisopropylbiphenyl (X-phos, 15 mg, 0.16 equiv), $Pd_2(dba)_3$ (14 mg, 0.08 equiv) and 3-nitro-aniline (41 mg, 1.5 equiv) were placed. The tube was evacuated and back-filled with argon (repeated three additional times). Then, 1 mL of degassed *t*-BuOH was introduced. The reaction mixture was allowed to stir at 100 °C for 15 h. After cooling to room temperature and quenching with water, the aqueous layer was extracted with AcOEt (4 × 60 mL). The combined organic layers were washed with brine (2 × 35 mL), dried over $MgSO_4$, filtered and the solvent was removed in vacuo. The crude product was purified by silica gel flash chromatography (PE/AcOEt 50:50–0:100 and then DCM/MeOH 90:10) providing the desired compound (57 mg) in 95% yield as a yellow solid. $C_{16}H_{11}N_5O_2$, 305.29 g mol^{-1}, ^{1}H NMR (400 MHz, DMSO) δ 9.92 (s, NH), 9.84 (s, 1H), 8.57 (t, J = 2.2 Hz, 1H), 8.26 (d, J = 8.0 Hz, 1H), 7.85 (dd, J = 8.1, 2.1 Hz, 1H), 7.81 (dd, J = 8.1, 2.2 Hz, 1H), 7.64 (d, J = 8.1 Hz, 1H), 7.60 (t, J = 8.2 Hz, 1H), 7.47 (t, J = 7.7 Hz, 1H), 7.30 (t, J = 7.6 Hz, 1H), 6.75 (d, J = 0.8 Hz, 1H); ^{13}C NMR (101 MHz, DMSO) δ 151.7 (C), 149.5 (C), 148.3 (C), 145.5 (C), 142.0 (C), 141.5 (CH), 130.2 (CH), 127.0 (C), 126.2 (CH), 124.9 (CH), 120.3 (CH), 117.8 (CH), 115.7 (CH), 112.7 (C), 111.8 (CH), 87.6 (CH); HRMS calcd for $C_{16}H_{12}N_5O_2$ $[M+H]^+$ 306.0986 found 306.0989.

3.1.7. *N*-(3-Methoxyphenyl)-benzo[4,5]imidazo[1,2-*c*]pyrimidin-3-ylamine (7)

In a Schlenk tube with a stirring bar, 3-chlorobenzo[4,5]imidazo[1,2-*c*]pyrimidine **1** (40 mg, 0.196 mmol), K_2CO_3 (81 mg, 3 equiv), 2-dicyclohexylphosphino-2′,4′,6′-triisopropylbiphenyl (X-phos, 15 mg, 0.16 equiv), and $Pd_2(dba)_3$ (14 mg, 0.08 equiv) were placed. The tube was evacuated and back-filled with argon (repeated three additional times). Then, 1 mL of degassed *t*-BuOH and *m*-anisidine (33 μL, 1.5 equiv) were introduced. The reaction mixture was allowed to stir at 100 °C for 15 h. After cooling to room temperature and quenching with water, the aqueous layer was extracted with DCM (4 × 30 mL). The combined organic layers were washed with brine (2 × 35 mL), dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure. The crude product was purified by silica gel flash chromatography (PE/AcOEt 50:50–20:80) providing the desired compound (37 mg) in 65% yield as a pale yellow solid. $C_{17}H_{14}N_4O$, 290.32 g mol^{-1}, ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.00 (s, 1H), 7.82 (d, J = 8.1 Hz, 1H), 7.75 (d, J = 8.1 Hz, 1H), 7.48 (t, J = 7.7 Hz, 1H), 7.33–7.22 (m, 2H), 6.95–6.83 (m, 4H), 6.70 (d, J = 8.2 Hz, 1H), 3.81 (s, OMe); ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.8 (C), 152.6 (C), 150.8 (C), 146.2 (C), 140.1 (C), 139.0 (CH), 130.5 (CH), 127.0 (C), 126.7 (CH), 120.9 (CH), 119.0 (CH), 113.7 (CH), 110.0 (CH), 109.9 (CH), 107.3 (CH), 85.7 (CH), 55.5 (CH_3); HRMS calcd for $C_{17}H_{14}N_4O$ $[M+H]^+$ 291.1240 found 291.1241.

3.1.8. 8-Bromo-3-chloro-benzo[4,5]imidazo[1,2-*c*]pyrimidine (8)

Under an inert atmosphere and in a 25 mL round-bottom flask, 3-chlorobenzo[4,5]imidazo[1,2-*c*]pyrimidine **1** (138 mg, 0.677 mmol) was suspended in 7 mL of anhydrous THF. *N*-Bromosuccinimide (180 mg, 1.5 equiv) was added and the mixture was stirred for 15 h at room temperature. Then, the reaction was quenched with a water/saturated aqueous $Na_2S_2O_3$ 1:1 mixture. The aqueous layer was extracted with AcOEt (60 mL, 2 × 20 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure. The crude product was purified by silica gel flash chromatography (DCM/AcOEt 100:0–97:3) to afford the brominated compound (130 mg) in 68% yield as a pale yellow solid. $C_{10}H_5BrClN_3$, 282.52 g mol^{-1}, ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.13 (d, J = 1.2 Hz, 1H), 8.16 (d, J = 1.7 Hz, 1H), 7.81 (d, J = 8.8 Hz, 1H), 7.72 (dd, J = 8.8, 1.8 Hz, 1H), 7.58 (d, J = 1.3 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 148.2 (C), 147.6 (C), 144.5 (C), 138.7 (CH), 131.3 (CH), 127.3 (C), 122.0 (CH), 116.2 (C), 114.1 (CH), 111.3 (CH); HRMS calcd for $C_{10}H_6BrClN_3$ $[M+H]^+$ 281.9428 found 281.9429.

3.1.9. *N*-Phenyl-(8-bromo-benzo[4,5]imidazo[1,2-*c*]pyrimidin-3-yl)amine (9)

In a Schlenk tube with a stirring bar, 8-bromo-3-chlorobenzo[4,5]imidazo[1,2-*c*]pyrimidine **8** (40 mg, 0.141 mmol), K_2CO_3 (59 mg, 3 equiv), 2-dicyclohexylphosphino-2′,4′,6′-triisopropylbiphenyl (X-phos, 11 mg, 0.16 equiv), and $Pd_2(dba)_3$ (10 mg, 0.08 equiv) were placed. The tube was evacuated and back-filled with argon (repeated three additional times). Then, 0.9 mL of *t*-BuOH and aniline (17 μL, 1.3 equiv) were introduced (degassed solvents were used). The reaction mixture was allowed to stir at 75 °C for 14 h. After cooling to room temperature and dilution with AcOEt, the mixture was filtered through a Celite® pad. The solvents of the filtrate were removed under reduced pressure. The crude product was purified by silica gel flash chromatography (DCM/AcOEt 100:0–95:5) to afford compound **9** in 16% yield (7.5 mg) as a pale yellow solid. $C_{16}H_{11}BrN_4$, 339.19 g mol^{-1}, ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.97 (d, J = 1.0 Hz, 1H), 7.87 (d, J = 1.5 Hz, 1H), 7.68 (d, J = 8.5 Hz, 1H), 7.41 (t, J = 9.2 Hz, 1H), 7.38 (dd, J = 8.5, 2.2 Hz, 1H), 7.32 (d, J = 7.6 Hz, 2H), 7.19 (t, J = 7.3 Hz, 1H), 6.79 (d, J = 1.1 Hz, 1H, NH); ^{13}C NMR (101 MHz, $CDCl_3$) δ 153.3 (C), 151.8 (C), 147.5 (C), 138.9 (CH), 138.5 (C), 129.8 (CH), 126.0 (C), 125.0 (CH), 123.8 (CH), 121.9 (CH), 121.8 (CH), 120.0 (C), 110.9 (CH), 85.0 (CH). MS (ESI) m/z 339.1, 341.0 $[M+H^+$; ^{79}Br, ^{81}Br].

3.1.10. 8-Bromo-3-(4-methoxyphenyl)-benzo[4,5]imidazo[1,2-*c*]pyrimidine (10)

In a Schlenk tube, under inert atmosphere, $Pd(Ph_3)_4$ (16 mg, 0.1 equiv), K_2CO_3 (59 mg, 3 equiv) and 4-methoxyphenylboronic acid (26 mg, 1.2 equiv) were added to a 0.05 M suspension of 8-bromo-3-chlorobenzo[4,5]imidazo[1,2-*c*]pyrimidine **8** (40 mg, 0.141 mmol) in 1,4-dioxane/H_2O 5:1 mixture. This mixture was stirred at 70 °C for 16 h. After cooling to rt and diluting with AcOEt, the mixture was filtered through a Celite® pad. The solvents were removed in vacuo. The crude product was purified by silica gel flash chromatography (DCM/AcOEt 100:0–90:10, then DCM/MeOH 98:2) to afford compound **10** in 9% yield (4.5 mg) as a pale yellow solid. $C_{17}H_{12}BrN_3O$, 354.20 g mol^{-1}, ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.30 (d, J = 1.4 Hz, 1H), 8.17 (d, J = 1.7 Hz, 1H), 8.09 (d, J = 8.9 Hz, 2H), 7.87 (d, J = 1.0 Hz, 1H), 7.81 (d, J = 8.5 Hz, 1H), 7.70 (dd, J = 8.7, 1.8 Hz, 1H), 7.05 (d, J = 8.9 Hz, 2H), 3.90 (s, 3H); MS (ESI) m/z 354.2, 356.2 $[M+H^+$; ^{79}Br, ^{81}Br].

3.1.11. 7-Bromo-4-chlorobenzo[4,5]imidazo[1,2-*a*]pyrazine (11)

Under an inert atmosphere and in a 10 mL round-bottom flask, 4-chlorobenzo[4,5]imidazo[1,2-*a*]pyrazine **2** (40 mg, 0.196 mmol) was suspended in 1 mL of anhydrous DCM. Bromine (20 μL, 2 equiv) was added dropwise and the mixture as allowed to stir for 16 h at room temperature. Then, the reaction was quenched with a water/saturated aqueous $Na_2S_2O_3$ 1:1 mixture. The aqueous

layer was extracted with DCM (4 × 20 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered and the solvent was removed in vacuo. The crude product was purified by silica gel flash chromatography (DCM/AcOEt 95:5) to afford the brominated compound (30 mg) in 54% yield as a white solid. $C_{10}H_5BrClN_3$, 282.52 g mol^{-1}, ^{1}H NMR (500 MHz, DMSO) δ 9.29 (s, 1H), 8.80 (d, J = 1.8 Hz, 1H), 8.18 (s, 1H), 8.02 (d, J = 8.9 Hz, 1H), 7.84 (dd, J = 8.9, 1.8 Hz, 1H); ^{13}C NMR (126 MHz, DMSO) δ 143.6 (C), 143.1 (CH), 142.8 (C), 130.2 (CH), 129.1 (C), 126.6 (CH), 125.7 (C), 122.6 (CH), 118.1 (CH), 115.3 (C); MS (ESI) m/z 282, 284 [M+H$^+$; ^{79}Br, ^{81}Br].

3.1.12. 4,7-Bis-[4-(4-methyl-piperazin-1-yl)-phenyl]-benzo[4,5]imidazo[1,2-*a*]pyrazine (12)

In a Schlenk tube, under inert atmosphere, $Pd(Ph_3)_4$ (10 mg, 0.1 equiv), K_2CO_3 (50 mg, 4 equiv) and 4-(4-methylpiperazin-1-yl)phenylboronic acid pinacol ester (81 mg, 3 equiv) were added to a 0.05 M suspension of 8-bromo-3-chlorobenzo[4,5]imidazo[1,2-*c*]pyrimidine **11** (25.4 mg, 0.0899 mmol) in 1,4-dioxane/H_2O 5:1 mixture. This mixture was stirred at 100 °C for 17 h. After cooling to rt and diluting with AcOEt, the mixture was filtered through a Celite® pad. The solvents were removed in vacuo. The crude product was purified by silica gel flash chromatography (DCM/MeOH 95:5–85:15) to afford compound **12** in 77% yield (35.9 mg) as a pale yellow solid. $C_{32}H_{35}N_7$, 517.67 g mol^{-1}, ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.21 (s, 1H), 8.00 (d, J = 8.7 Hz, 1H), 7.80–7.73 (m, 2H), 7.49 (d, J = 8.6 Hz, 2H), 7.31 (d, J = 8.7 Hz, 2H), 7.13 (d, J = 0.8 Hz, 1H), 7.10 (d, J = 8.7 Hz, 2H), 6.91 (d, J = 8.7 Hz, 2H), 3.43–3.35 (m, 4H), 3.28–3.20 (m, 4H), 2.67–2.61 (m, 4H), 2.61–2.57 (m, 4H), 2.40 (s, 3H), 2.37 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 152.7 (C), 150.5 (C), 143.9 (C), 143.7 (CH), 143.5 (C), 135.3 (C), 134.3 (C), 131.8 (C), 130.4 (CH), 129.1 (s), 127.8 (CH), 127.7 (CH), 126.0 (CH), 121.1 (CH), 121.1 (s), 116.1 (CH), 115.4 (CH), 112.4 (CH), 55.1 (CH_2), 55.0 (CH_2), 48.8 (CH_2), 48.3 (CH_2), 46.3 (CH_2), 46.2 (CH_2); HRMS calcd for $C_{32}H_{36}N_7$ [M+H]$^+$ 518.3027 found 518.3010.

3.1.13. 4,7-Dichlorobenzo[4,5]imidazo[1,2-*a*]pyrazine (13)

Under an inert atmosphere and in a 25 mL round-bottom flask, 4-chlorobenzo[4,5]imidazo[1,2-*a*]pyrazine **2** (82.5 mg, 0.405 mmol) was dissolved in 4 mL of anhydrous DMF. *N*-Chlorosuccinimide (86 mg, 1.6 equiv) was added and the mixture was stirred for 21 h at room temperature. Then, the reaction was quenched with water (30 mL), saturated $NaHCO_3$ solution (10 mL), and brine (10 mL). The aqueous layer was extracted with AcOEt (4 × 15 mL). The combined organic layers were washed with a water/brine 1:1 mixture (3 × 30 mL), dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure. The crude product was purified by silica gel flash chromatography (DCM/AcOEt 95:5–85:15) to provide the di-halogenated compound (53 mg) in 54% yield as a white solid. $C_{10}H_5Cl_2N_3$, 238.07 g mol^{-1}, ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.16 (s, 1H), 8.54 (d, J = 1.9 Hz, 1H), 7.96 (d, J = 8.9 Hz, 1H), 7.94 (s, 1H), 7.60 (dd, J = 8.9, 2.0 Hz, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 143.9 (C), 143.4 (CH), 143.0 (C), 129.4 (C), 128.9 (C), 128.5 (CH), 127.0 (CH), 125.6 (C), 122.4 (CH), 115.4 (CH); MS (ESI) m/z = 238.2 [M+H]$^+$.

3.1.14. 7-Chloro-4-(4-methoxyphenyl)-benzo[4,5]imidazo[1,2-*a*]pyrazine (14)

In a Schlenk tube, under inert atmosphere, $Pd(Ph_3)_4$ (42 mg, 0.1 equiv), K_2CO_3 (150 mg, 3 equiv) and 4-methoxyphenyl boronic acid (66 mg, 1.2 equiv) were added to a 0.04 M suspension of 4,7-dichlorobenzo[4,5]imidazo[1,2-*a*]pyrazine **13** (86 mg, 0.36 mmol) in THF/H_2O 5:1 mixture (9 mL). This mixture was stirred at 70 °C for 16 h. After cooling to rt and diluting with AcOEt, the mixture was filtered through a Celite® pad. The solvents were removed in vacuo. The crude product was purified by silica gel flash chromatography (DCM/AcOEt 75:25–60:40), followed by a trituration in MeOH and filtration. The desired product was the solid obtained by trituration in 86% yield (96 mg) as a pale yellow solid. $C_{17}H_{12}ClN_3O$, 309.75 g mol^{-1}, ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.26 (s, 1H), 7.95 (d, J = 8.9 Hz, 1H), 7.78 (s, 1H), 7.52 (d, J = 8.7 Hz, 2H), 7.50 (dd, J = 8.6, 2.2 Hz, 1H), 7.16 (d, J = 8.8 Hz, 2H), 6.94 (d, J = 1.7 Hz, 1H), 3.97 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.7 (C), 144.3 (CH), 143.6 (C), 143.4 (C), 133.8 (C), 130.7 (CH), 128.7 (C), 128.3 (CH), 128.2 (C), 127.7 (CH), 122.7 (C), 122.3 (CH), 115.1 (CH), 115.0 (CH), 55.7 (CH_3). MS (ESI) m/z = 310.2 [M+H]$^+$, 641.1 [2M+Na]$^+$.

3.1.15. 7-Chloro-4-(*E*)-styryl-benzo[4,5]imidazo[1,2-*a*]pyrazine (15)

In a Schlenk tube, under inert atmosphere, $Pd(Ph_3)_4$ (24 mg, 0.1 equiv), K_2CO_3 (85 mg, 3 equiv) and (*E*)-styrylboronic acid (39 mg, 1.3 equiv) were added to a 0.04 M suspension of 4,7-dichlorobenzo[4,5]imidazo[1,2-*a*]pyrazine **13** (49 mg, 0.206 mmol) in THF/H_2O 5:1 mixture (5 mL). This mixture was stirred at 70 °C for 16 h. After cooling to rt and diluting with AcOEt, the mixture was filtered through a Celite® pad. The solvents were removed under reduced pressure. The crude product was purified by silica gel flash chromatography (DCM/AcOEt 90:10–70:30), followed by a trituration in MeOH and filtration. The desired product was the solid obtained by trituration in 67% yield (42.5 mg) as a yellow solid. $C_{18}H_{12}ClN_3$, 305.76 g mol^{-1}, ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.17 (s, 1H), 8.05 (d, J = 1.7 Hz, 1H), 8.02 (s, 1H), 7.96 (d, J = 8.9 Hz, 1H), 7.65 (d, J = 7.2 Hz, 2H), 7.55 (dd, J = 8.9, 1.9 Hz, 1H), 7.50 (d, J = 15.1 Hz, 1H), 7.49 (t, J = 7.3 Hz, 2H), 7.43 (t, J = 7.3 Hz, 1H), 7.36 (d, J = 15.9 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 143.8 (CH), 143.4 (C), 143.1 (C), 137.6 (CH), 135.2 (C), 133.3 (C), 130.0 (CH), 129.4 (CH), 129.0 (C), 128.9 (C), 127.7 (CH), 127.6 (CH), 126.1 (CH), 122.4 (CH), 117.0 (CH), 114.7 (CH); MS (ESI) m/z = 306.2 [M+H]$^+$.

3.1.16. *N*-(4-[4-(4-Methoxyphenyl)-benzo[4,5]imidazo[1,2-*a*]pyrazin-7-yl]phenyl)acetamide (16)

In a Schlenk tube with a stirring bar, 7-chloro-4-(4-methoxyphenyl)benzo[4,5]imidazo[1,2-*a*]pyrazine **14** (36 mg, 0.116 mmol), K_3PO_4 (74 mg, 3 equiv), (4-acetamidophenyl)boronic acid (41 mg, 2 equiv), 2-dicyclohexylphosphino-2′,6′-dimethoxybiphenyl (S-phos, 9.5 mg, 0.2 equiv) and $Pd(OAc)_2$ (2.6 mg, 0.1 equiv) were placed. The tube was evacuated and back-filled with argon (repeated three additional times). Then, 2.3 mL of degassed *n*-butanol was introduced to obtain a 0.05 M suspension. The reaction mixture was allowed to stir at 100 °C for 16 h. After cooling to room temperature and dilution with AcOEt, the mixture was filtered through a Celite® pad. The solvents of the filtrate were removed in vacuo. The crude product was purified by silica gel flash chromatography (DCM/MeOH 98:2–97:3) to provide the desired product in 80% yield (38 mg) as a pale yellow solid. $C_{25}H_{20}N_4O_2$, 408.45 g mol^{-1}, ^{1}H NMR (400 MHz, MeOD) δ 9.17 (s, 1H), 7.98 (d, J = 8.7 Hz, 1H), 7.81 (dd, J = 10.1 Hz, J = 1.5 Hz, 1H), 7.80 (s, 1H), 7.59 (s, 1H), 7.59 (d, J = 8.7 Hz, 2H), 7.56 (d, J = 8.6 Hz, 2H), 7.31 (d, J = 8.6 Hz, 2H), 7.20 (d, J = 8.7 Hz, 2H), 7.03 (s, 1H), 3.95 (s, 3H), 2.14 (s, 3H); ^{13}C NMR (101 MHz, MeOD) δ 171.0 (C), 162.6 (C), 144.0 (C), 143.8 (C), 143.6 (CH), 138.7 (C), 136.8 (C), 136.6 (C), 135.3 (C), 131.5 (CH), 129.5 (C), 128.2 (CH), 128.0 (CH), 127.4 (CH), 123.4 (C), 121.2 (CH), 121.0 (CH), 115.5 (CH), 113.6 (CH), 56.0 (CH_3), 23.9 (CH_3); HRMS calcd for $C_{25}H_{21}N_4O_2$ [M+H]$^+$ 409.1659 found 409.1656.

3.1.17. 4-(4-Methoxyphenyl)-7-[4-(4-methyl-piperazin-1-yl)-phenyl]benzo[4,5]imidazo[1,2-*a*]pyrazine (17)

In a Schlenk tube with a stirring bar, 7-chloro-4-(4-methoxyphenyl)benzo[4,5]imidazo[1,2-*a*]pyrazine **14** (35 mg, 0.113 mmol), K_3PO_4 (74 mg, 3 equiv), 4-(4-methylpiperazin-1-yl)phenylboronic

acid pinacol ester (68 mg, 2 equiv), 2-dicyclohexylphosphino-2′,6′-dimethoxybiphenyl (S-Phos, 9.5 mg, 0.2 equiv) and $Pd(OAc)_2$ (2.6 mg, 0.1 equiv) were placed. The tube was evacuated and back-filled with argon (repeated three additional times). Then, 2.3 mL of degassed *n*-butanol were introduced to obtain a 0.05 M suspension. The reaction mixture was allowed to stir at 100 °C for 14 h. After cooling to room temperature and dilution with AcOEt, the mixture was filtered through a Celite® pad. The solvents of the filtrate were removed under reduced pressure. The crude product was purified by silica gel flash chromatography (DCM/MeOH 98:2–92:8) to provide the desired product in 40% yield (20 mg) as a light orange solid. $C_{28}H_{27}N_5O$, 449.55 g mol^{-1}, ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.25 (s, 1H), 8.03 (d, J = 8.7 Hz, 1H), 7.78 (dd, J = 8.1, 2.2 Hz, 1H), 7.77 (s, 1H), 7.57 (d, J = 8.7 Hz, 2H), 7.31 (d, J = 8.7 Hz, 2H), 7.16 (d, J = 8.7 Hz, 2H), 7.08 (d, J = 1.1 Hz, 1H), 6.94 (d, J = 8.8 Hz, 2H), 3.95 (s, 3H), 3.31–3.20 (m, 4H), 2.67–2.56 (m, 4H), 2.38 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.6 (C), 150.5 (C), 144.1 (CH), 143.9 (C), 143.4 (C), 135.8 (C), 133.9 (C), 131.9 (C), 130.9 (CH), 129.1 (C), 128.0 (CH), 127.9 (CH), 126.3 (CH), 123.4 (C), 121.3 (CH), 116.2 (CH), 114.8 (CH), 112.3 (CH), 55.8 (CH_3), 55.0 (CH_2), 48.7 (CH_2), 46.1 (CH_3); HRMS calcd for $C_{28}H_{28}N_5O$ $[M+H]^+$ 450.2288 found 450.2270.

3.1.18. 4-(4-Methoxyphenyl)-7-(*E*)-styryl-benzo[4,5]imidazo[1,2-*a*]pyrazine (18)

In a Schlenk tube with a stirring bar, 7-chloro-4-(4-methoxyphenyl)benzo[4,5]imidazo[1,2-*a*]pyrazine **14** (32 mg, 0.103 mmol), K_3PO_4 (66 mg, 3 equiv), (*E*)-styrylboronic acid (30.5 mg, 2 equiv), 2-dicyclohexylphosphino-2′,6′-dimethoxybiphenyl (S-Phos, 8.5 mg, 0.2 equiv) and $Pd(OAc)_2$ (2.3 mg, 0.1 equiv) were placed. The tube was evacuated and back-filled with argon (repeated three additional times). Then, 2.2 mL of degassed *n*-butanol was introduced to obtain a 0.05 M suspension. The reaction mixture was allowed to stir at 100 °C for 14 h. After cooling to room temperature and dilution with AcOEt, the mixture was filtered through a Celite® pad. The solvents of the filtrate were removed in vacuo. The crude product was purified by silica gel flash chromatography (DCM/MeOH 98:2), followed by a trituration in MeOH and filtration. The desired product was the solid obtained by trituration in 52% yield (20.2 mg) as a yellow solid. $C_{25}H_{19}N_3O$, 377.44 g mol^{-1}, ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.25 (s, 1H), 7.99 (d, J = 8.7 Hz, 1H), 7.79 (s, 1H), 7.76 (dd, J = 8.7, 1.5 Hz, 1H), 7.57 (d, J = 8.7 Hz, 2H), 7.45 (d, J = 7.3 Hz, 2H), 7.35 (t, J = 7.6 Hz, 2H), 7.28–7.24 (m, 1H), 7.20 (d, J = 8.7 Hz, 2H), 6.99 (d, J = 16.1 Hz, 1H), 6.97 (d, J = 0.6 Hz, 1H), 6.88 (d, J = 16.3 Hz, 1H), 4.00 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.65 (C), 144.82 (C), 144.18 (CH), 143.65 (C), 137.20 (C), 133.90 (C), 132.37 (C), 130.94 (CH), 128.99 (CH), 128.98 (C), 128.86 (CH), 128.68 (CH), 128.16 (CH), 127.90 (CH), 126.57 (CH), 125.58 (CH), 123.40 (C), 121.40 (CH), 114.87 (CH), 113.04 (CH), 55.78 (CH_3); HRMS calcd for $C_{25}H_{20}N_3O$ $[M+H]^+$ 378.1601 found 378.1613.

3.1.19. 4-(*E*)-Styryl-7-(4-methoxyphenyl)-benzo[4,5]imidazo[1,2-*a*]pyrazine (19)

In a Schlenk tube with a stirring bar, 7-chloro-4-(*E*)-styryl-benzo[4,5]imidazo[1,2-*a*]pyrazine **15** (33 mg, 0.108 mmol), K_3PO_4 (69 mg, 3 equiv), 4-methoxyphenyl boronic acid (33 mg, 2 equiv), 2-dicyclohexylphosphino-2′,6′-dimethoxybiphenyl (S-phos, 8.9 mg, 0.2 equiv) and $Pd(OAc)_2$ (2.4 mg, 0.1 equiv) were placed. The tube was evacuated and back-filled with argon (repeated three additional times). Then, 2.2 mL of degassed *n*-butanol were introduced to obtain a 0.05 M suspension. The reaction mixture was allowed to stir at 100 °C for 14 h. After cooling to room temperature and dilution with AcOEt, the mixture was filtered through a Celite® pad. The solvents of the filtrate were removed under reduced pressure. The crude product was purified by silica gel flash chromatography (DCM/MeOH 99:1–98:2), followed by a trituration in MeOH and filtration. The desired compound was the solid obtained by trituration in 41% yield (17 mg) as a yellow solid. $C_{25}H_{19}N_3O$, 377.44 g mol^{-1}, ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.20 (s, 1H), 8.25 (s, 1H), 8.09 (d, J = 8.7 Hz, 1H), 8.04 (s, 1H), 7.84 (dd, J = 8.7, 1.4 Hz, 1H), 7.69 (d, J = 16.1 Hz, 1H), 7.66 (d, J = 7.2 Hz, 2H), 7.56 (d, J = 8.7 Hz, 2H), 7.48 (t, J = 7.3 Hz, 2H), 7.45–7.41 (m, 1H), 7.38 (d, J = 16.1 Hz, 1H), 6.99 (d, J = 8.7 Hz, 2H), 3.86 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 159.5 (C), 144.1 (C), 143.7 (CH), 143.1 (C), 137.0 (CH), 136.8 (C), 135.5 (C), 133.7 (C), 133.5 (C), 129.8 (CH), 129.6 (C), 129.4 (CH), 128.6 (CH), 127.5 (CH), 126.8 (CH), 125.9 (CH), 121.6 (CH), 117.8 (CH), 114.7 (CH), 112.5 (CH), 55.5 (CH_3); HRMS calcd for $C_{25}H_{20}N_3O$ $[M+H]^+$ 378.1601 found 378.1588.

3.2. Biological assays

Recombinant wild-type and L1196M mutant ALK catalytic domains were expressed in the Baculovirus system, purified by affinity chromatography and used in the ELISA-based colorimetric kinase assay as described previously.[15] Cell growth inhibition was tested in a [^{3}H]-thymidine incorporation assay, as described.[18] Kinase activity or cell-associated radioactivity were plotted as a function of inhibitor concentration, using GraphPad software. The concentration that causes 50% enzyme inhibition compared to the DMSO-treated control was recorded (IC_{50}). Detailed procedures are provided in the Supplementary material.

3.3. Molecular docking

The 3D-coordinates of the wild type human Anaplastic Lymphoma Kinase (ALK) catalytic domain in complex with the inhibitor NVP-TAE684 was retrieved from the Protein Data Bank (PDB 2XB7)[16] and energetically minimized in the internal coordinate space with Molsoft ICM v3.7-3d.[19] Molecular docking was performed using a ICM-VLS (Molsoft ICM) protocol validated by assessing that redocking of NVP-TAE684 into the X-ray structure (PDB 2XB7) devoid of ligand reproduces the experimentally determined binding mode. This protocol includes that the binding site is represented by five types of interaction potentials: (i) van der Waals potential for a hydrogen atom probe; (ii) van der Waals potential for a heavy-atom probe (generic carbon of 1.7 Å radius); (iii) optimized electrostatic term; (iv) hydrophobic terms; and (v) loan-pair-based potential, which reflects directional preferences in hydrogen bonding. The energy terms are based on the Merck Molecular Force Field (MMRF) to account for solvation free energy and entropic contribution.[20] Modified inter-molecular terms such as soft van der Waals and hydrogen-bonding, as well as a hydrophobic, term are added. Conformational sampling is based on the biased probability Monte Carlo (BPMC) procedure, which randomly selects a conformation in the internal coordinate space and then makes a step to a new random position independent of the previous one, but according to a predefined continuous probability distribution. It has also been shown that after each random step, full local minimization greatly improves the efficiency of the procedure. In the ICM-VLS (Molsoft ICM) screening procedure, the ligand scoring is optimized to obtain maximal separation between the binders and non-binders. Each compound is assigned a score according to its fit within the receptor; this ICM score accounts for continuum and discreet electrostatics, hydrophobicity and entropy parameters.[20,21]

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Supplementary data

Supplementary data (general experimental procedures, biochemical assay procedures, inhibition data at different ATP concentrations, and criteria of purity (^{1}H and ^{13}C NMR spectra, melting points, and HPLC chromatograms)) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2014.01.007.

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