Discovery of Novel and Highly Selective Cyclopropane ALK Inhibitors through a Fragment-Assisted, Structure-Based Drug Design

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ABSTRACT: Fragment screening is frequently used for hit identification. However, there was no report starting from a small fragment for the development of an anaplastic lymphoma kinase (ALK) inhibitor, despite the number of ALK inhibitors reported. We began our research with the fragment hit F-1 and our subsequent linker design, and its docking analysis yielded novel cis-1,2,2-trisubstituted cyclopropane 1. The fragment information was integrated with a structure-based approach to improve upon the selectivity over tropomyosin receptor kinase A, leading to the potent and highly selective ALK inhibitor, 4-trifluoromethylphenoxy-cis-1,2,2-trisubstituted cyclopropane 12. This work shows that fragments become a powerful tool for both lead generation and optimization, such as the improvement of selectivity, by combining them with a structure-based drug design approach, resulting in the fast and efficient development of a novel, potent, and highly selective compound.

INTRODUCTION

Fragment-based drug design (FBDD) was reported first as "structure—activity relationship (SAR) by NMR" by Fesik et al. at Abbott Pharmaceutical in 1996.1 During the past 2 decades, various kinds of FBDD approaches have been reported. In hit identification, fragment screening has the advantage over high-throughput screening (HTS) because it generally has higher hit rates, and the hit compounds from fragment screening tend to show good solubility and high affinity with low molecular weight.2 Also for hit identification, the fragment is a powerful tool in the lead generation process. Several methods are reported for fragment-based lead generation, for example, fragment-based growing and linking,3 molecular hybridization,4 and scaffold hopping.5 In the optimization phase, a cocrystal structure of a compound with a target protein can be obtained more easily than in the lead generation step because of the high affinity of the compound; therefore, a structure-based drug design (SBDD) is predominantly adopted in that phase. As for anaplastic lymphoma kinase (ALK) inhibitors, there had been no report on the use of a fragment-based approach for the development of an ALK inhibitor, despite the number of them in use for the treatment of ALK-positive nonsmall cell lung cancer (NSCLC),6−10 brain metastasis ALK-positive lung cancer,10,11 and central nervous system (CNS) disorders.10,12 All of the reports began with HTS or virtual screening13 and used SBDD in the optimization process.10 In this work, we describe the discovery process of cyclopropane derivatives as novel, potent, and highly selective ALK inhibitors from a small fragment. We also show that a fragment-assisted approach is effective in improving not only activity but also selectivity in lead optimization.

RESULTS AND DISCUSSION

Fragment Identification and Analysis of Cocrystal Structures. We began our studies with high-concentration 
(0.3−300 μM) biochemical assays of a small fragment library. Among those fragments, 3-(3-methyl-1H-pyrazol-5-yl)pyridine (F-1) had a half-maximal inhibitory concentration (IC50) value
of 220 μM with a high ligand efficiency (LE) \([-\Delta G/\text{heavy atom count (HAC), defined as the free energy of binding divided by the number of nonhydrogen heavy atoms}^\text{14}\] of 0.42. The cocrystal structure of fragment F-1 with the ALK protein shown in Figure 1A (PDB code 7JYT) suggested that the pyrazole moiety of this fragment acted as the "hinge" binder by making two hydrogen bonds; one was formed between the pyrazole NH and Glu1197 O=C and the other between the pyrazole nitrogen with amide NH of Met1199. The pyridine moiety of fragment F-1 made the CH–π interaction with Gly1202 (Figure 1A). Crizotinib is the first launched ALK inhibitor for the treatment of NSCLC, and its ALK inhibitory activity was highly potent under our enzymatic and cellular assays with IC\(_{50}\) values of 1.8 and 37 nM, respectively. X-ray crystallographic analysis of the cocrystal structure of crizotinib with ALK revealed several key interactions (Figure 1B, PDB code 2XP2). The 2-aminopyridine NH and pyridine nitrogen form two hydrogen bonds to the protein backbone of the kinase hinge region. The pyrazole ring makes a CH–π interaction with the Gly1202 (3.7 Å), and the 2,6-dichloro-3-fluorophenyl group contributes to the potency as a hydrophobic interaction, in which fluorine at C3 plays a crucial role by filling a small hydrophobic pocket, formed by the Gly1269 and Gln1254 residues, and polarizing the adjacent C4–H bond to productively interact with Arg1253 carbonyl oxygen. The superposition of the cocrystal structure of fragment lead F-1/ALK on that of crizotinib/ALK (Figure 1C) implied that fragment F-1 can be connected to the 6-chlorine atom on the benzene ring of crizotinib at the 4-position of the pyridine ring of F-1 with the shortest distance. The distance between 4-C on the pyridine ring of F-1 and 6-Cl on the benzene ring of crizotinib was 3.88 Å and corresponded to the three-bond length.

**Design of Novel Scaffold.** According to an analysis of the binding mode of crizotinib in the ALK protein, the biaryl structure and the lipophilic benzene ring were considered to be important to the high binding affinity. Therefore, we planned to connect the fragment hit F-1 with the lipophilic benzene ring in the following three steps: (1) identification of the attachment point, (2) determination of the length of a linker based on the distance, and (3) design of the linkers.

The design for the novel scaffold began by setting the oxygen atom at the 4-position of the pyridine ring at the 4-position of the pyridine ring in order to provide the attachment point of the linker. Although a biaryl structure is normally twisted by the introduction of a substituent at the ortho-position,\(^\text{15}\) we assumed that the oxygen atom at the ortho-position might assist to keep the two rings, the pyrazole ring and the pyridine ring, coplanar by forming an intramolecular hydrogen bond with the pyrazole NH or the pyrazole CH. An unchanging conformation was thought to be necessary to maintain the potent kinase inhibitory activity because the angle of the pyridine ring was important to form a CH–π interaction with the Gly1202. In addition, we replaced the chlorine atom at the 6-position of crizotinib with a carbon atom in order to provide an attachment point on the aryl moiety. As a second step, the
2,6-dichloro-3-fluorobenzene group was simplified into a 4-fluorobenzene ring to make the calculation simple. As mentioned earlier, the distance between the pyridine ring of fragment F-1 and the chlorine atom of crizotinib was 3.88 Å; thus, the distance between the oxygen atom and the replaced carbon atom was 2.7 Å, which corresponded to the two-bond-length linkers. In the last phase, we exhaustively designed the two-bond-length linkers to restrict the conformations by cyclization (Figure 2). We expected the cyclization to be particularly effective on an adjustment of the angle of the 4-fluorobenzene ring.

Ethyl and cyclized linkers were designed as two-bond-length linkers. The ring size in the cyclization ranged from a three-membered ring to a six-membered ring, and each cyclized linker had four stereoisomers; therefore, 17 structures were generated in total. The linkers were named designs D-1 to D-17 as shown in Figure 3.

Computational Evaluation of Designed Compounds.
In order to select the best scaffold, we performed a docking analysis of the designed compounds using the software packages Maestro and Glide SP (Schrödinger Inc). For the docking analysis, we calculated three scores: ΔE, root-mean-square deviation (rmsd), and ΔG. The ΔE values express the conformational energy penalties (ΔE) possessed by each design compound in the ALK protein. The rmsds are the measures of the average distances between the benzene ring of crizotinib in the cocrystal structure with the ALK and the benzene ring of D-1 to D-17. The rmsd value is usually used to
assess how well a submitted structure matches the known target structure. Thus, a lower rmsd value indicates a better fit of the designed compound to crizotinib. The ΔG values indicate the sum of the binding energies, such as hydrogen bonding, van der Waals forces, and electrostatic, hydrophobic, and solvation contributions to binding.

The results of the calculations for all the designed linkers (D-1 to D-17) are summarized in Table 1. It has been reported that if the conformational energy penalties (ΔE) of the ligand are >3 kcal/mol, the calculated conformations would be uncertain. Thus, we set the cutoff value of ΔE as 3 kcal/mol. According to the cutoff value of ΔE, D-1, D-2, D-3, D-4, D-5, D-8, and D-12 were selected. Among the seven compounds, D-1, D-3, D-8, and D-12 showed similarly low rmsd (<1 Å) and ΔG values. Last, we synthesized cis-1,2-cyclopropane D-3, regarding it as the best compound based on the following two reasons: (1) the binding of compound D-3 is entropically favored over those of compounds D-1 and D-12 because the conformation of D-3 is more rigid and (2) the synthetic tractability of D-3 was better than that of D-8.

**Fragment-Assisted SAR Analysis.** Before synthesizing the designed cis-1,2-cyclopropane derivative, we prepared structurally related fragments to the initial hit F-1 by combinatorial chemistry. The SAR around the pyridine ring of the fragment F-1 may be rapidly and easily analyzed using the additional fragment library, which can be prepared in just two steps: a Suzuki coupling and a deprotection of the tetrahydropyranyl (THP) others group. The second fragment library consisted of mono-substituted phenyl groups at ortho, meta, or para positions and heteroaromatic rings such as pyrimidine (Figure 4).

A high-concentration assay against the second fragment library was conducted. The conversion into other heteroaromatic rings, such as 2-pyridine or 5-pyrimidine, did not improve the potency. The introduction of substituents at the 2- and 4-positions also diminished the potency compared with the initial fragment hit 1 (data not shown), whereas the substituents at the 3-position had a great impact on the ALK inhibitory activity. The results are shown in Table 2. The electron-withdrawing groups at the 3-position tended to increase the ALK inhibitory activities (F-5, F-7, and F-9). Among them, the 3-cyano-phenyl fragment F-7 showed the most potent ALK inhibitory activity with an IC_{50} of 51 μM. Because 3- and 4-pyridine fragments F-1 and F-2 were more potent than the phenyl fragment F-3, it was expected that the introduction of the cyano group on the pyridine fragment might show better potency compared with fragment F-7. However, the combination of a cyano group with a 4-pyridine ring resulted in a decrease in ALK inhibitory activity (F-13, IC_{50} = 130 μM). Consequently, we decided to incorporate the selected scaffold, cis-1,2-cyclopropane, into the 3-cyano-phenyl fragment F-7.

**Evaluation of Designed Compounds.** The installation of the cis-1,2-cycloprooxy group on the 3-cyano phenyl fragment F-7 generated 5-cyano-2-cycloprooxy derivative 1 and 3-cyano-2-cycloprooxy derivative 2 as shown in Figure 5A; therefore, it was necessary to discuss the direction of the cyano group at the meta-position of the benzene ring in the pocket of ALK protein because of its asymmetry. We compared the co-crystal structure of fragment F-1/ALK with a newly obtained cocrystal structure of fragment F-5/ALK (Figure 5B). In the two co-crystal structures, both the chloro group and the pyridine nitrogen pointed toward the E0 region. It was to be expected that the chlorine atom could interact with the hydrophobic residues Ala1200 and Gly1201 in the E0 region; however, it was interesting that the polar pyridine nitrogen also sat in the hydrophobic E0 region. On the basis of the observations, it was hypothesized that the hydrophilic cyano group might also point toward the E0 region and the 5-

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**Table 1. Docking Scores of Designs 1–17**

| design no. | ΔE (kcal) | rmsd (Å) | ΔG (kcal) |
|------------|-----------|---------|-----------|
| D-1        | 1.42      | 0.32    | -47.6     |
| D-2        | 2.87      | 1.38    | -45.5     |
| D-3        | 2.19      | 0.34    | -48.4     |
| D-4        | 1.30      | 3.58    | -41.5     |
| D-5        | 2.05      | 6.39    | -41.5     |
| D-6        | 3.90      | 1.56    | -46.8     |
| D-7        | 8.67      | 1.79    | -42.2     |
| D-8        | 1.39      | 0.74    | -51.0     |
| D-9        | 7.37      | 3.11    | -39.9     |
| D-10       | 3.84      | 1.48    | -47.5     |
| D-11       | 3.98      | 0.29    | -48.9     |
| D-12       | 2.44      | 0.53    | -49.1     |
| D-13       | 11.6      | 2.44    | -41.5     |
| D-14       | 4.31      | 2.03    | -47.4     |
| D-15       | 4.54      | 0.35    | -47.3     |
| D-16       | 14.8      | 1.83    | -32.2     |
| D-17       | 4.69      | 1.78    | -37.8     |

*All calculations were performed by MAESTRO and Glide SP.*

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**Table 2. Results of Fragment Screening at High Concentration (0.3–300 μM)**

| compd (T-no) | Ar         | ALK IC_{50} (μM)* |
|--------------|------------|------------------|
| F-1          | 3-pyridine | 220 (170–290)   |
| F-2          | 4-pyridine | 240 (200–290)   |
| F-3          | Ph         | >300             |
| F-4          | 3-F-Ph     | 240 (170–350)   |
| F-5          | 3-Cl-Ph    | 66 (49–88)      |
| F-6          | 3-Me-Ph    | 230 (160–330)   |
| F-7          | 3-CN-Ph    | 51 (40–66)      |
| F-8          | 3-MeO-Ph   | 170 (130–220)   |
| F-9          | 3-CF_{3}-Ph| 60 (47–78)      |
| F-10         | 3-ClBu-Ph  | 270 (230–310)   |
| F-11         | 3-CF_{2}O-Ph| 95 (77–120)    |
| F-12         | 3-MeSO_{2}-Ph| 120 (95–160) |
| F-13         | 3-CN-4-pyridine| 130 (100–160)|

*IC_{50} values and 95% confidence intervals (CIs) (given in parentheses) were calculated by nonlinear regression analysis of percent inhibition data (n = 2).
cyano-2-cyclopropoxy derivative would properly reflect the fragment activity. Therefore, we synthesized a chiral (1S,2S)-2-(2,4-difluorophenyl)cyclopropoxy-4-cyano-benzene (1) to evaluate the profiles of the novel scaffold. We referred our recent report that explored the SAR of substitutions on the fluoro benzene ring because the binding mode of the benzene ring in the designed compound was estimated to be similar to that of the benzene ring in the reported 1H-pyrrolo[2,3-b]pyridine analogues. We replaced 4-fluorobenzene in the designed compound into 2,4-difluorobenzene that showed the strongest ALK inhibitory activity among the various substituted benzene or pyridine, although the data was not shown in the report. The 4-fluorobenzene ring was utilized only in the simulation to simplify the calculations due to its symmetry.

As expected from the calculations, the chiral (1S,2S)-2-(2,4-difluorophenyl)cyclopropoxy-4-cyano-benzene derivative (1) showed potent ALK enzymatic inhibitory activity with an IC$_{50}$ value of 0.080 μM and more than 30-fold greater selectivity over 37 kinases in a corporate kinase panel assay while exhibiting low ALK cellular inhibitory activity with an IC$_{50}$ value of >1.0 μM and moderate tropomyosin receptor kinase (Trk)A inhibitory activity (Table 3). The LE value of compound 1 for ALK was also remarkably high at 0.37, demonstrating that compound 1 was an attractive lead compound. On the other hand, the enantiomer 3 corresponding to D-2 exhibited a 16-fold decrease in ALK inhibitory activity with an IC$_{50}$ value of 1.3 μM. The absolute configuration of compound 1 was determined as the 1S,2S form by X-ray single diffraction analysis after derivatization to aminomethyl compound 4 as shown in Figure 6. The assay results of 1 and 3 were consistent with the calculations for D-2 and D-3.

The cocrystal structure of compound 1 with ALK was solved and superimposed with the structure of crizotinib extracted from the cocrystal structure with ALK (Figure 7A). As expected from the binding mode of fragment F-1, compound 1 also bound to the ALK in the adenosine triphosphate pocket, and the pyrazole nitrogen and NH of compound 1 made hydrogen bonds with the backbone carbonyl of hinge residue Glu1197 and the amide NH of hinge residue Met1199. As expected, the observed coplanarity of the biaryl structure in the cocrystal supported our strategy 1 in Figure 2. In addition, compound 1 also formed several key interactions. The cyanobenzene ring was interacting with a hydrophobic residue, Gly1202. Leu1256 participated in two CH−π interactions with the 2,4-difluorophenyl group and the methyl pyrazole ring, leading to the L-shape conformation that is thought to be important to ALK potency.

Table 3. Enzymatic Kinase Activity Profiles of 1 and 3

| compd | stereo chemistry | IC$_{50}$ (μM)$^{a}$ | IC$_{50}$ (μM)$^{b}$ | IC$_{50}$ (μM)$^{c}$ | LE$^{b}$ | rmsd (Å) | ΔE (kcal) |
|-------|------------------|---------------------|---------------------|---------------------|---------|----------|-----------|
| 1     | 1S, 2S           | 0.08                | >1.0                | 0.95                | 0.37    | 0.747    | 1.08      |
| 3     | 1R, 2R           | 1.3                 | NT$^{d}$            | NT$^{d}$            | 0.31    | 1.62     | 1.86      |

$^{a}$IC$_{50}$ values and 95% CIs (given in parentheses) were calculated by nonlinear regression analysis of percent inhibition data (n = 2). $^{b}$LE (ligand efficiency) = −1.36 log IC$_{50}$/number of heavy atoms. $^{c}$Not tested.

Figure 6. ORTEP of compound 4. Thermal ellipsoids are drawn at 30% probability.
respectively, the equivalent LE of 0.37, and the improved ligand lipophilicity efficiency of 4.3 compared to the nonsubstituted compound 5, and had the best selectivity, being a 17-fold increase over TrkA.

**Fragment-Assisted Kinase Selectivity Improvement.** Next, we demonstrated a design for the improvement of the kinase selectivity using fragment information. The selectivity of the Trk family (TrkA, TrkB, and TrkC) in ALK programs had already been surpassed in several reports. The reported inhibitors utilized the single difference of the amino acid residue between ALK and the Trk family. The ALK residue Leu1198 was replaced by a tyrosine from the Trk family (Figure 8A). The residues were close to the cyano group of compound 1 according to the cocrystal structure of 1/ALK.

The fragment assay results shown in Table 3 reminded us of the trifluoromethyl benzene fragment F-8, whose ALK inhibitory activity was 60 μM, similar to F-7 at 51 μM (Figure 8B). As for fragment activity, the trifluoromethylphenoxy cyclopropane derivative was expected to present nearly equivalent ALK inhibitory activity to cyanophenoxy cyclopropane derivative 9. Conversion from a cyano group into a trifluoromethyl group was also predicted to improve the selectivity over TrkA based on two factors: electronic and steric aspects. First, it is generally recognized that the trifluoromethyl group is highly electronegative, thereby leading to electronic repulsion with a polar amino acid residue represented by a tyrosine. Second, it is considered that the trifluoromethyl group is sterically bulkier than the cyano group, as supported by a web-based calculation using the freely available Molinspiration online service, with a gap in volumes of the substituents of 14.4 Å. The fact provided an opportunity for the bulkier trifluoromethyl group to clash with a tyrosine in the Trk family. The trifluoromethylphenoxy cyclopropane derivative 12 was synthesized and evaluated.

The profiles of compound 12 are summarized in Figure 9. As speculated, compound 12 showed potent ALK enzyme inhibitory activity with an IC₅₀ value of 0.018 μM and a 128-fold increase in selectivity over TrkA but unexpectedly exhibited the decreased ALK cellular inhibitory activity with an IC₅₀ value of 0.95 μM. Compound 12 was chiral, and the absolute configuration was determined as 1S,2S form by X-ray single diffraction analysis after the derivatization to a p-nitrosulfonamide compound 13. We confirmed that 1S,2S compound 12 was the eutomer because the other enantiomer 1R,2R exhibited a weaker ALK enzymatic inhibitory activity with an IC₅₀ value of 0.40 μM. A cocrystal structure of 12

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**Table 4. SAR of Substituents at the 2-Position of Cyclopropane Derivatives**

| compd | R     | stereo chemistry | ALK enzyme IC₅₀ (μM) | ALK cell IC₅₀ (μM) | TrkA enzyme IC₅₀ (μM) | LE b for ALK | LLE c for ALK |
|-------|-------|------------------|----------------------|-------------------|-----------------------|-------------|--------------|
| 1     | H     | 1S, 2S           | 0.080                | >1.0              | 0.95                  | 0.37        | 2.9          |
| 5     | H     | racemic          | 0.13                 | NT⁴               | 1.2                   | 0.36        | 2.7          |
| 6     | CO₂Me | racemic          | 0.041                | 0.45              | 0.085                 | 0.34        | 3.3          |
| 7     | CH₂OH | racemic          | 0.034                | NT⁴               | 0.14                  | 0.37        | 4.3          |
| 8     | CH₂OMe| racemic          | 0.043                | 0.89              | 0.38                  | 0.35        | 3.4          |
| 9     | CH₂NH₂| racemic          | 0.029                | 0.16              | 0.50                  | 0.37        | 4.3          |
| 10    | CH₂NHMe| racemic       | 0.049               | NT⁴               | 0.92                  | 0.35        | 3.8          |
| 11    | CH₂NMe₂| racemic        | 0.11                 | NT⁴               | 1.6                   | 0.32        | 2.8          |

⁴IC₅₀ values and 95% CIs (given in parentheses) were calculated by nonlinear regression analysis of percent inhibition data (n = 2). ⁵LE (ligand efficiency) = −1.36 log IC₅₀/number of heavy atoms. ⁶LLE = pIC₅₀ − c log P. ⁷Not tested.
bound to ALK was obtained and indicated almost the same binding mode as that of compound 1. Interestingly, the amino group of 12 forms a direct hydrogen bond with His1124 in the p-loop and a water-mediated hydrogen bond network involving in Asn1254, contributing to an increase in the binding affinity. A KINOMEscan screening platform of 456 kinases, using an active site-directed competition binding assay, revealed that compound 12 inhibited only five kinases other than ALK, LTK and ULK3 with 5–10% control, DCAMKL1, FAK, and ULK1 with 10–35% control at 300 nM compound concentration, demonstrating remarkably high kinase selectivity, whereas compound 12 also inhibited mutated ALK (L1198M) with 10–35% control at 300 nM, and ALK (C1156Y) with 1–5% control at 300 nM.

■ CHEMISTRY

Scheme 1 shows the synthesis of 4-cyanophenoxy-cis-1,2,2-disubstituted cyclopropane derivatives 1 and 3. A commercially available 2-bromo-4-cyanophenol 14 was converted into vinyl ether 15 with an iridium catalyst. The vinyl ether moiety was coupled with tosylhydrazone 22 separately prepared from aldehyde 20 with a rhodium catalyst to afford cyclopropane derivative 16. The methylpyrazole moiety was introduced by Suzuki reaction with boronic acid pinacol ester 25, followed by the deprotection of the THP group to afford racemic cyclopropane compound 17. Enantiomers 18 (tR1) and 19 (tR2) were obtained by a preparative chiral high-performance liquid chromatography (HPLC) separation of racemic compound 17 using a CHIRALCEL OJ column. The three free compounds, 17, 18, and 19, were converted to hydrochloride salts 5, 1, and 3, respectively. The chiral aminobenzyl compound 4 was prepared from the chiral compound 18 via reduction of the cyano group. The tosylhydrazone 22 was prepared from commercially available benzaldehyde 20 and tosylhydrazine 21. The pyrazole boronic ester 25 was prepared in a two-step reaction: THP protection and regioselective borylation from the commercially available material 23.

Scheme 2 shows the synthesis of 4-cyanophenoxy-cis-1,2,2-trisubstituted cyclopropane derivatives 6–11. Vinyl ether 15 was converted to the trisubstituted cyclopropane 26 by diazo compound 34 and a catalytic amount of rhodium acetate. The methylpyrazole moiety was introduced by Suzuki reaction with pyrazole boronic ester 25 to afford the intermediate 27. The ester 27 was selectively reduced to alcohol intermediate 28 by calcium borohydride generated in situ under the coexistence of the cyano group. The hydroxy group of 28 was methylated by sodium hydride and iodomethane to afford methoxyintermediate 29. The primary amine 30 was prepared by Mitsunobu reaction with phthalimide, followed by deprotection with hydrazine. Secondary amine 31 and tertiary amine 32 were prepared from alcohol intermediate 28 in two steps: (1) Dess–Martin oxidation and (2) reductive amination. The deprotection reaction of the THP group of 27–32 by acid afforded 4-cyanophenoxy-cis-1,2,2-trisubstituted cyclopropane derivatives 6–11. The diazo compound 34 was prepared from 2,4-difluorophenyl acetate 33 by diazo transfer reaction under basic conditions.

Scheme 3 shows the synthesis of 4-trifluoromethylphenoxy-cis-1,2,2-trisubstituted cyclopropane derivatives 12 and 13. The vinyl ether intermediate 36 was prepared by the same method, as shown in Scheme 1. The cyclopropanation followed by the introduction of the pyrazole moiety was conducted under the same condition in Scheme 2 to afford the cyclopropyl ester intermediate 38. The ester was reduced to afford the alcohol 39 by lithium aluminum hydride (LAH). The racemic primary amino compound 40 was synthesized through the same reactions in Scheme 2. The optical resolution of compound 40 afforded the chiral amine 41 and its enantiomer 42. The chiral amine 41 was converted to salt form 12 by hydrogen chloride. The sulfonamide 13 was prepared by sultonylation reaction from the chiral amino compound 41.

■ CONCLUSIONS

Several ALK inhibitors were developed and approved as potential treatments for ALK-positive cancers such as NSCLC. These studies began with hit compounds with relatively large molecular weights, identified from their own corporate libraries, and utilized SBDD approaches for the optimization of activity and selectivity. However, there was no report adopting a fragment-based approach for an ALK program. In this study, we have described the strategic design of a novel scaffold from small fragments and the efficient improvement of both potency and kinase selectivity using fragment information. We began our research with a high concentration assay of the fragment library, and the subsequent linker design and its docking analysis afforded a novel cis-1,2,2-trisubstituted cyclopropane structure with potent ALK inhibitory activity. Furthermore, the fragment information was integrated with a structure-based approach to overcome the selectivity over...
TrkA. The resulting compound 12 showed potent ALK inhibitory activity and remarkably high kinase selectivity. However, the compound 12 traded off the ALK cellular inhibitory activity against the selectivity over TrkA. Further optimization to increase the cellular activity, such as an introduction of the solubilizing moiety like a piperidine of crizotinib, and subsequent in vivo evaluation will be required for developing a tool of the pharmacological evaluation.

A fragment-based approach is often used to generate the lead compound in the development of a new chemotype by connecting two fragments, optimizing the fragment itself, or adding functional groups onto the fragment. This work indicates that a fragment-based approach becomes a powerful tool for selectivity, even in the optimization phase, by combining it with an SBDD approach. This mixed approach could also make the process simple and rational and, eventually, could contribute to the development of a novel, potent, and highly selective compound, in a short period, without synthesizing a large number of compounds. This approach can be a useful tool for chemotype hopping in various kinase projects in cases where there is a cocrystal structure of the known ligand with the target protein.

**EXPERIMENTAL SECTION**

**General Methods.** General Chemistry Information. All solvents and reagents were obtained from commercial sources and were used as received. Yields were not optimized. All reactions were monitored by thin layer chromatography analysis on Merck Kieselgel 60 F254 plates or Fuji Silysia NH plates or liquid chromatography–mass spectrometry (LC–MS) analysis. LC–MS analysis was performed on a Shimadzu liquid chromatography–mass spectrometer system.
operating in the APCI (+ or −) or ESI (+ or −) ionization mode. Analytes were eluted using a linear gradient of 0.05% trifluoroacetic acid (TFA) containing water/acetonitrile or 5 mM ammonium acetate containing the water/acetonitrile mobile phase and detected at 220 nm. Column chromatography was carried out on silica gel ([Merck Kieselgel 60, 70–230 mesh, Merck] or [Chromatorex NH-DM 1020, 100–200 mesh, Fuji Silysia Chemical, Ltd.]) or on prepacked Purif-Pack columns (SI or NH, particle size: 60 μm, Fuji Silysia Chemical, Ltd.) or on a chromatography column (either silica gel or NH, particle size: 60 μm, Fuji Silysia Chemical, Ltd.). Analytical HPLC was performed with Corona Charged Aerosol Detector (CAD) or a photodiode array detector. The column was a Capcell Pak C18AQ (50 mm × 3.0 mm i.d., Shiseido, Japan) or L-column 2 ODS (30 mm × 2.0 mm i.d., CERI, Japan) with a temperature of 50 °C and a flow rate of 0.5 mL/min. Mobile phases A and B under neutral conditions were a mixture of 50 mmol/L ammonium acetate, water, and acetonitrile (1:8:1, v/v/v) and a mixture of 50 mmol/L ammonium acetate and acetonitrile (1:9, v/v), respectively. The ratio of mobile phase B was increased linearly from 5 to 95% over 3 min, 95% over the next 1 min. Mobile phases A and B under acidic conditions were a mixture of 0.2% formic acid in 10 mmol/L ammonium formate and 0.2% formic acid in acetonitrile, respectively. The ratio of mobile phase B was increased linearly from 14 to 86% over 3 min and 86% over the next 1 min. Elemental analyses were carried out by Takeda Analytical Laboratories, and the results were within ±0.4% of theoretical values. All final test compounds were purified to >95% chemical purity as measured by analytical HPLC. Proton nuclear magnetic resonance (1H NMR) spectra were in all cases consistent with the proposed structures. 1H NMR spectra

Scheme 1. Synthesis of 4-Cyanophenoxy-cis-1,2-disubstituted Cyclopropane Derivatives

Reagents and conditions: (a) vinyl acetate, [Ir(1,5-cod)Cl]2, Na2CO3, toluene, 100 °C, 92%; (b) 22, Rh2(OAc)4, LHMDS, BnEt3NCl, dioxane, 40 °C, and 30%; (c) 25, Pd(PPh3)4, Na2CO3, DME−H2O, and reflux; (d) 4 N HCl/EtOAc, EtOAc, 0 °C, and 84% in two steps; (e) optical resolution; (f) LAH, THF, 50 °C, and 89%; (g) MeOH, rt, and 89%; (h) dihydropyrane, TFA, toluene, reflux, and 96%; and (i) n-BuLi, triisopropyl borate, THF, −78 °C, then pinacol, acetic acid, rt, and 67%.
were recorded on a Varian Mercury-300 (300 MHz) or a Bruker DPX300 (300 MHz) instrument. All proton shifts are given in parts per million (ppm) downfield from tetramethysilane (δ) as the internal standard in a deuterated solvent, and coupling constants (J) are in hertz (Hz). NMR data are reported as follows: chemical shift, integration, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; quint, quintet; m, multiplet; dd, doublet of doublets; td, triplet of doublets; ddd, doublet of doublet of doublets; and brs, broad singlet), and coupling constants.

4-(((1S,2S)-2-(2,4-Difluorophenyl)cyclopropyl)oxy)-3-(5-methyl-1H-pyrazol-3-yl)benzonitrile Hydrochloride (1). To a solution of 18 (30 mg, 0.09 mmol) in EtOAc (0.5 mL) was added 4 N HCl in EtOAc (0.5 mL, 2.00 mmol) at room temperature. The mixture was concentrated in vacuo. The resulted crystals were washed with EtOAc and dried in vacuo to afford 1 (23.5 mg, 0.061 mmol, 71%) as a colorless crystal. 1H NMR (600 MHz, DMSO-d6, 300 K): δ 1.45 (1H, td, J = 7.2, 3.3 Hz), 1.49 (1H, dt, J = 9.5, 6.2 Hz), 2.12 (3H, s), 2.52−2.56 (1H, m), 4.37 (1H, td, J = 6.4, 3.3 Hz), 5.51 (1H, s), 6.93 (1H, td, J = 8.5, 2.4 Hz), 7.25 (1H, dd, J = 10.5, 9.4, 2.6 Hz), 7.28−7.34 (1H, m), 7.53 (1H, d, J = 8.8 Hz), 7.78 (1H, dd, J = 8.6, 2.0 Hz), 8.12 (1H, d, J = 2.2 Hz), 2H not detected. 13C NMR (151 MHz, DMSO-d6, 300 K): δ 10.8, 10.6, 15.7 (d, J = 3.9 Hz), 55.4, 103.3 (t, J = 26.0 Hz), 103.5, 105.1, 110.8 (dd, J = 20.7, 3.6 Hz), 114.5, 118.8, 119.6 (dd, J = 14.9, 3.3 Hz), 31993

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Scheme 2. Synthesis of 4-Cyanophenoxy-cis-1,2,2-trisubstituted Cyclopropane Derivatives

Reagents and conditions: (a) 34, Rh2(OAc)4, CH2Cl2, 45 °C, and 49%; (b) 25, Pd(PPh3)4, Na2CO3, DME−H2O, reflux, and quant.; (c) NaBH4, CaCl2, EtOH−THF, 0 °C to rt, and 73%; (d) NaH, MeI, THF, rt, and 75%; (e) phthalimidem, Ph3P, DIAD, THF, and 0 °C to rt; (f) hydrazine monohydrate, EtOH, rt, and 89% in two steps; (g) Dess−Martin reagent, MeCN, and 0 °C; (h) amines, NaBH(OAc)3, TEA, MeOH, rt, and 69−100% in two steps; (i) 4 N HCl/EtOAc or 2 N HCl/MeOH, MeOH, 0 °C, and 49−98%; and (j) 4-AcNHPhSO2N3, DBU, MeCN, 0 °C to rt, and 94%.
Scheme 3. Synthesis of 4-Trifluoromethylphenoxy-cis-1,2,2-trisubstituted Cyclopropane Derivatives

Reagents and conditions: (a) vinyl acetate, [Ir(1,5-cod)Cl]2, Na2CO3, toluene, 100 °C, and 53%; (b) 34, Rh2(OAc)4, Et2O, rt, and 62%; (c) 25, Pd(PPh3)4, Na2CO3, DME−H2O, reflux, and 80%; (d) LAH, THF, 0 °C, and quant.; (e) phthalimide, Ph3P, DIAD, THF, and 0 °C to rt; (f) hydrazine monohydrate, EtOH, and rt; (g) 2 N HCl/MeOH, EtOAc, rt, and 38% in three steps; (h) optical resolution; (i) 4 N HCl/EtOAc, MeOH, rt, and 97%; and (j) p-NsCl, pyridine, rt, and 54%.

The NMR spectrum was obtained with the racemic compound.

4-(((1R,2R)-2-(2,4-Difluorophenyl)cyclopropyl)oxy)-3-(5-methyl-1H-pyrazol-3-yl)benzonitrile Hydrochloride (35).

4-((1S,2S)-2-(2,4-Difluorophenyl)cyclopropanyl)-3-(5-methyl-1H-pyrazol-3-yl)phenyl)methanamine (40).

121.7, 130.8 (dd, J = 9.7, 5.3 Hz), 130.9, 132.7, 140.8, 142.4, 158.1, 161.0 (dd, J = 244.6, 11.9 Hz), 161.9 (dd, J = 247.1, 12.7 Hz). MS (ESI/APCI) m/z: 352.0 [M + H]+. mp 198−206 °C; Anal. Calcd for C20H16N3OClF2: C, 61.94; H, 4.16; N, 10.84. Found: C, 61.87; H, 4.18; N, 10.76. HPLC purity: 100%. 99.7% ee.
temperature, diluted with THF, and then quenched with water (0.046 mL), 15% NaOH aq (0.046 mL), and water (0.138 mL). The mixture was stirred overnight and filtered by a Celite pad. The filtrate was concentrated in vacuo to afford 4 (95.0 mg, 0.268 mmol, 89%) as a pale yellow gum. \(^1\)H NMR (300 MHz, DMSO-\(d_6\)): \(\delta\) 1.37–1.56 (2H, m), 2.09 (3H, s), 2.52–2.55 (1H, m), 4.36 (1H, td, \(J = 6.2, 3.4\) Hz), 5.44 (1H, s), 6.93 (1H, td, \(J = 8.4, 2.3\) Hz), 7.20–7.39 (2H, m), 7.51 (1H, d, \(J = 8.6, 2.2\) Hz), 8.10 (1H, d, \(J = 2.2\) Hz), 2H not detected. MS (ESI/APCI) \(m/z\): 352.0 [M + H]\(^+\). mp 198–206 °C. HPLC purity: 100%.

**Methyl (1'R,2'R)-2-(4-Cyano-2-(5-methyl-1H-pyrazol-3-yl)phenoxy)-1-(2,4-difluorophenyl)cyclopropanecarboxylate Hydrochloride (6).** To a solution of 27 (146 mg, 0.30 mmol) in MeOH (3 mL) was added 4 N HCl in EtOAc (0.50 mL, 2.00 mmol) at 0 °C. After stirring for 10 min, the reaction was concentrated in vacuo. The resulted solid was washed with EtOAc and dried in vacuo to afford 6 (130 mg, 0.291 mmol, 98%) as colorless crystals. \(^1\)H NMR (300 MHz, DMSO-\(d_6\)): \(\delta\) 1.93 (1H, dd, \(J = 6.6, 4.3\) Hz), 2.06 (1H, t, \(J = 6.4\) Hz), 2.10 (3H, s), 3.64 (3H, s), 4.84 (1H, dd, \(J = 6.3, 4.2\) Hz), 5.21 (1H, s), 7.06 (1H, tt, \(J = 8.6, 1.3\) Hz), 7.32 (1H, ddd, \(J = 10.4, 9.3, 2.6\) Hz), 7.49 (1H, td, \(J = 8.6, 6.4\) Hz), 7.62 (1H, d, \(J = 8.7\) Hz), 7.78 (1H, dd, \(J = 8.7, 2.3\) Hz), 8.07 (1H, d, \(J = 2.1\) Hz), 2H not detected. MS (ESI/APCI) \(m/z\): 410.1 [M + H]\(^+\). mp 1860–1890 °C. Anal. Calcd for C\(_{32}\)H\(_{25}\)N\(_4\)O\(_3\).ClF\(_2\): C, 59.27; H, 4.07; N, 9.42. Found: C, 59.17; H, 4.32; N, 9.70. HPLC purity: 99.0%.

**4-(((1'R,2'R)-2-(2,4-Difluorophenyl)-2-(hydroxymethyl)cyclopropyl)oxy)-3-(5-methyl-1H-pyrazol-3-yl)benzonitrile Hydrochloride (7).** To a solution of 28 (69.8 mg, 0.150 mmol) in MeOH (3 mL) was added 2 N HCl in MeOH (0.5 mL, 1.00 mmol) at 0 °C. After stirring for 10 min, the reaction mixture was concentrated in vacuo. The resulted solid was recrystallized in EtOAc-EtOH. The crystals were collected by filtration and dried in vacuo to afford 7 (35.2 mg, 0.084 mmol, 56%) as colorless crystals. \(^1\)H NMR (300 MHz, DMSO-\(d_6\)): \(\delta\) 1.09 (1H, dd, \(J = 6.3, 3.1\) Hz), 1.54 (1H, t, \(J = 6.1\) Hz), 2.10 (3H, s), 3.46 (1H, d, \(J = 11.3\) Hz), 3.72 (1H, d, \(J = 11.3\) Hz), 4.32 (1H, dd, \(J = 5.9, 3.1\) Hz), 5.35 (1H, d, \(J = 0.6\) Hz), 7.04 (1H, tt, \(J = 8.5, 1.4\) Hz), 7.27 (1H, ddd, \(J = 10.7, 9.3, 2.6\) Hz), 7.43 (1H, td, \(J = 8.6, 6.8\) Hz), 7.60 (1H, d, \(J = 8.7\) Hz), 7.79 (1H, dd, \(J = 8.6, 2.2\) Hz), 8.07 (1H, d, \(J = 2.1\) Hz), 3H not detected. MS (ESI/APCI) \(m/z\): 380.1 [M + H]\(^+\). mp 195–200 °C. Anal. Calcd for C\(_{23}\)H\(_{19}\)N\(_2\)O\(_2\).ClF\(_2\): C, 60.36; H, 4.43; N, 10.06. Found: C, 60.39; H, 4.38; N, 9.98. HPLC purity: 100%.
A mixture of 14 (5.20 g, 263 mmol), vinyl acetate (7.26 mL, 78.8 mmol), and Ti(i-Bu)4 (39.6 mg, 0.065 mmol, 54%) as a pale yellow oil. 1H NMR (300 MHz, DMSO-d6): δ 0.374 mmol, 30%) as a colorless oil. 1H NMR (300 MHz, DMSO-d6): δ 10.48 (1H, d, J = 10.4, 2.4 Hz), 7.00 (1H, d, J = 13.4, 2.1 Hz), 7.00 (1H, d, J = 13.4, 9.5 Hz), 7.36 (1H, d, J = 8.3 Hz), 7.86–7.93 (1H, m), 8.26 (1H, d, J = 1.9 Hz).

3-Bromo-4-((vinyl)benzonitrile) (15). A mixture of 14 (5.20 g, 263 mmol), vinyl acetate (7.26 mL, 78.8 mmol), and Ti(i-Bu)4 (39.6 mg, 0.065 mmol, 54%) as a pale yellow oil. 1H NMR (300 MHz, DMSO-d6): δ 0.374 mmol, 30%) as a colorless oil. 1H NMR (300 MHz, DMSO-d6): δ 10.48 (1H, d, J = 10.4, 2.4 Hz), 7.00 (1H, d, J = 13.4, 2.1 Hz), 7.00 (1H, d, J = 13.4, 9.5 Hz), 7.36 (1H, d, J = 8.3 Hz), 7.86–7.93 (1H, m), 8.26 (1H, d, J = 1.9 Hz).

3-Bromo-4-(((1R5,2RS)-2-(2,4-difluorophenyl)cyclopropyl)oxy)benzonitrile (16). To a solution of 22 (0.388 g, 1.25 mmol) in dry THF (5 mL) was added LHMDS (1.31 mL, 1.31 mmol) at −78 °C under the Ar atmosphere. The mixture was stirred at the same temperature for 15 min, gradually warmed to room temperature, and concentrated in vacuo. The residue was mixed with diethyl ether and diethyl ether (6 mL) and filtered and concentrated in vacuo. The residue was crystallized in hexane—EtOAc. The crystals were collected by filtration and dried in vacuo to afford 22 (2.77 g, 8.93 mmol, 89%) as a colorless crystal. 1H NMR (300 MHz, DMSO-d6): δ 3.71 (3H, s), 7.31 (1H, td, J = 8.6, 2.4 Hz), 7.32 (1H, d, J = 11.3, 9.1, 2.5 Hz), 7.41 (2H, d, J = 7.9 Hz), 7.67–7.82 (3H, m), 8.02 (1H, s), 11.63 (1H, br s).

3-Methyl-1-((tetrahydro-2H-pyran-2-yl)-1H-pyrazole (24). To a mixture of 3-methyl-1H-pyrazole 23 (25.0 g, 304 mmol) in toluene (152 mL) were added 3,4-dihydro-2H-pyran (30.6 mL, 335 mmol) and TFA (1.17 mL, 15.2 mmol) at room temperature. The mixture was stirred under reflux for 1 day. The mixture was cooled with brine and NaHCO3 aq at room temperature, cooled with brine and NaHCO3 aq at room temperature, concentrated in vacuo, and purified by column chromatography (silica gel, hexane/ethyl acetate, 100:0 to 78:2) to afford 24 (48.5 g, 292 mmol, 96%) as a yellow oil. 1H NMR (300 MHz, DMSO-d6): δ 1.40–1.73 (3H, m), 1.78–2.11 (3H, m), 2.15 (3H, s), 3.52–3.69 (1H, m), 3.80–3.96 (1H, m), 5.26 (1H, d, J = 10.4, 2.4 Hz), 6.06 (1H, d, J = 2.3 Hz), 7.30 (1H, d, J = 2.3 Hz).

3-Methyl-1-((tetrahydro-2H-pyran-2-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole (25). To a solution of 24 (23.0 g, 138 mmol) in dry THF (173 mL) was added n-BuLi (91 mL, 145 mmol) dropwise at −78 °C. The reaction mixture was stirred at the same temperature for 2 h. The mixture was cooled with brine and NaHCO3 aq at room temperature, cooled with brine and NaHCO3 aq at room temperature, concentrated in vacuo, and purified by column chromatography (silica gel, hexane/ethyl acetate, 100:0 to 78:2) to afford 25 (48.5 g, 292 mmol, 96%) as a yellow oil. 1H NMR (300 MHz, DMSO-d6): δ 1.40–1.73 (3H, m), 1.78–2.11 (3H, m), 2.15 (3H, s), 3.52–3.69 (1H, m), 3.80–3.96 (1H, m), 5.26 (1H, d, J = 10.4, 2.4 Hz), 6.06 (1H, d, J = 2.3 Hz), 7.30 (1H, d, J = 2.3 Hz).

3-Methyl-1-((tetrahydro-2H-pyran-2-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole (25). To a solution of 24 (23.0 g, 138 mmol) in dry THF (173 mL) was added n-BuLi (91 mL, 145 mmol) dropwise at −78 °C. The reaction mixture was stirred at the same temperature for 2 h. The mixture was cooled with brine and NaHCO3 aq at room temperature, cooled with brine and NaHCO3 aq at room temperature, concentrated in vacuo, and purified by column chromatography (silica gel, hexane/ethyl acetate, 100:0 to 78:2) to afford 25 (48.5 g, 292 mmol, 96%) as a yellow oil. 1H NMR (300 MHz, DMSO-d6): δ 1.40–1.73 (3H, m), 1.78–2.11 (3H, m), 2.15 (3H, s), 3.52–3.69 (1H, m), 3.80–3.96 (1H, m), 5.26 (1H, d, J = 10.4, 2.4 Hz), 6.06 (1H, d, J = 2.3 Hz), 7.30 (1H, d, J = 2.3 Hz).

3-Methyl-1-((tetrahydro-2H-pyran-2-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole (25). To a solution of 24 (23.0 g, 138 mmol) in dry THF (173 mL) was added n-BuLi (91 mL, 145 mmol) dropwise at −78 °C. The reaction mixture was stirred at the same temperature for 2 h. The mixture was cooled with brine and NaHCO3 aq at room temperature, cooled with brine and NaHCO3 aq at room temperature, concentrated in vacuo, and purified by column chromatography (silica gel, hexane/ethyl acetate, 100:0 to 78:2) to afford 25 (48.5 g, 292 mmol, 96%) as a yellow oil. 1H NMR (300 MHz, DMSO-d6): δ 1.40–1.73 (3H, m), 1.78–2.11 (3H, m), 2.15 (3H, s), 3.52–3.69 (1H, m), 3.80–3.96 (1H, m), 5.26 (1H, d, J = 10.4, 2.4 Hz), 6.06 (1H, d, J = 2.3 Hz), 7.70 (1H, d, J = 2.3 Hz).

Final characterization data for 1H-pyrazole 25: 31996
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acetate, 98:2 to 85:15) to a purified cis product. The residue was diluted with water and extracted with EtOAc twice. The combined organic layers were washed with brine, dried over MgSO₄, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, hexane/ethyl acetate, 100:0 to 85:15) to a prepurified cis product. The residue was purified by column chromatography (silica gel, hexane/ethyl acetate, 100:0 to 85:15) to a purified cis product. The residue was diluted with water and extracted with EtOAc twice. The combined organic layers were washed with brine, dried over MgSO₄, and concentrated in vacuo.

Methyl (1RS,2RS)-2-(2-Bromo-4-cyanophenyl)cyclopropenecarboxylate (26). To a mixture of 15 (3.33 g, 14.42 mmol) and rhodium(II) acetate dimer (0.064 g, 0.14 mmol) in CH₂Cl₂ (30 mL) was added dropwise a solution of 34 (4.28 g, 20.22 mmol) in CH₂Cl₂ (10 mL) at 45 °C. The reaction mixture was stirred at room temperature and monitored by GC. The residue was purified by column chromatography (silica gel, hexane/ethyl acetate, 98:2 to 85:15) to afford 26 (2.89 g, 7.08 mmol, 49%) as colorless crystals. 1H NMR (300 MHz, DMSO-d₆): δ 1.82 (1H, dd, J = 6.7, 4.3 Hz), 2.07 (1H, t, J = 6.7 Hz), 3.63 (3H, s), 4.90 (1H, dd, J = 6.5, 4.3 Hz), 6.98–7.10 (1H, m), 7.19 (1H, ddd, J = 10.4, 9.4, 2.6 Hz), 7.46 (1H, td, J = 8.6, 6.6 Hz), 7.55 (1H, d, J = 8.6 Hz), 7.88 (1H, dd, J = 8.6, 2.0 Hz), 8.04 (1H, d, J = 2.0 Hz).

Methyl (1RS,2SR)-2-(4-Cyano-2-(3-methyl-1-(tetrahydro-2H-pyran-2-yl)-1H-pyrrol-5-yl)phenoxo)-1-(2,4-difluorophenyl)cyclopropenecarboxylate (27). The title compound was prepared in a yield comparable to a colorless amorphous powder from 26 using the procedure analogous to that described for the synthesis of 17. 1H NMR (300 MHz, DMSO-d₆): δ 1.33–1.70 (4H, m), 1.70–1.80 (1H, m), 1.80–1.95 (1H, m), 2.00–2.14 (4H, m), 3.21–3.38 (1H, m), 3.61 (3H, s), 3.77 (1H, d, J = 11.4 Hz), 4.68–4.86 (2H, m), 5.06–5.49 (1H, m), 6.84–7.19 (2H, m), 7.32 (2H, qd, J = 8.9, 6.5 Hz), 7.58–7.68 (2H, m), 7.95 (1H, dd, J = 8.7, 2.2 Hz). MS (ESI/APCI) m/z: 492 [M + H]+.

4-(((1RS,2RS)-2-(2,4-Difluorophenyl)-2-(hydroxymethyl)cyclopropenecarboxyloxy)-3-(3-methyl-1-(tetrahydro-2H-pyran-2-yl)-1H-pyrrol-5-yl)benzonitrile (28). To a suspension of calcium hydride (26.9 g, 92.0 mmol, 67%) in 1,4-dioxane (0.493 g, 1.00 mmol) in dry THF (1.5 mL) was added diisopropyl azodicarboxylate (DIAD) (0.789 mL, 1.50 mmol) and then iodomethane (100 mL, 9.15 mmol) at room temperature. The mixture was stirred at 0 °C for 15 min, iodomethane was added thereto, and the mixture was allowed to gradually warm to room temperature, and stirred for 2 h and then concentrated in vacuo. The residue was diluted with water and extracted with EtOAc twice. The combined organic layers were washed with sat. brine, dried over MgSO₄, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, hexane/ethyl acetate, 85:15 to 70:30) to afford 29 (77.0 mg, 0.160 mmol, 75%) as a colorless gum. 1H NMR (300 MHz, DMSO-d₆): δ 1.01–1.14 (1H, m), 1.30–1.95 (6H, m), 2.01–2.36 (4H, m), 3.15–3.22 (3H, m), 3.22–3.38 (2H, m), 3.49–3.65 (1H, m), 3.76 (1H, dd, J = 11.0 Hz), 4.23–4.36 (1H, m), 4.62–4.88 (1H, m), 5.10–5.59 (1H, m), 6.77–6.99 (1H, m), 6.99–7.15 (1H, m), 7.25 (1H, dd, J = 12.7, 8.7, 6.8 Hz), 7.59 (1H, dd, J = 8.7, 1.8 Hz), 7.65 (1H, dd, J = 3.7, 2.2 Hz), 7.96 (1H, dt, J = 8.7, 1.8 Hz). MS (ESI/APCI) m/z: 482 [M + H]+.

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colorless amorphous powder. To a mixture of aldehyde intermediate (0.250 g, 0.540 mmol) and methylamine (0.275 mL, 2.70 mmol) in MeOH (2.5 mL) was added NaBH(OAc)₃ (0.572 g, 2.70 mmol) at room temperature. After stirring overnight, the mixture was concentrated in vacuo. The residue was partitioned between EtOAc and water. The separated aqueous layer was extracted with EtOAc again. The combined organic layers were washed with sat. NaHCO₃ aq and sat. brine, dried over MgSO₄, and concentrated in vacuo. The residue was purified by column chromatography (NH-silica gel, hexane/ethyl acetate, 100:0 to 1:3). 

CH₃CN (35 mL) was added 1,8-diazabicyclo[5.4.0]undec-7-ene (2.16 (10H, m), 2.76 (1H, d), J = 3.4 Hz), 3.07 (1H, m), 3.21 (1H, d, J = 11.4 Hz), 4.10 (1H, m), 4.24 (1H, m), 4.63-4.90 (1H, m), 5.05-5.57 (1H, m), 6.76-7.14 (2H, m), 7.14-7.32 (1H, m), 7.59 (1H, d, J = 8.8 Hz), 7.64 (1H, dd, J = 3.9, 2.1 Hz), 7.89-8.00 (1H, m). 

MS (ESI/APCI) m/z: 479.3 [M + H]⁺.

**Methyl Diazo(2,4-difluorophenyl)acetate (34).** To a solution of a benzyl ether (3.28 g, 13.2 mmol) and 4-acetamidobenzenesulfonyl azide (3.48 g, 14.5 mmol) in CH₂CN (35 mL) was added 1,6-diazabicyclo[5.4.0]undec-7-ene (DBU) (2.36 mL, 15.8 mmol) at 0 °C. The reaction mixture was gradually warmed to room temperature, stirred for 1 h, and concentrated in vacuo. The residue was dissolved in EtOAc and washed with water. The separated aqueous layer was extracted with EtOAc again. The combined organic layers were washed with sat. brine, dried over MgSO₄, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, hexane/ethyl acetate, 10:0 to 90:10) to afford 34 (2.62 g, 12.4 mmol, 94%) as an orange oil.

**Calculation Conditions.** The docking study was performed using the protein structure of the arcocrystal structure of ALK in the Protein Data Bank (PDB ID 2XP2). The protein structure was prepared by the Protein Preparation Workflow in Maestro 11.5 (version 11.5.001, Schrödinger, New York, USA) remaining all crystallographic waters and the disorder loops were constructed by the 3D structures of designed compounds from D-1 to D-17 were built with the default parameters by LigPrep 4.0 version. The docking study was performed with the default parameters by using Glide SP 7.3 version. The grid-enclosing box was placed on the centroid of the ligand based on the crystal structures of ALK. In this docking study, the position of the methylpyrazole moiety was restricted to one of the arcocrystal structure of ALK in complex.
with F-1 within 0.5 Å rmsd tolerance of the reference core. The conformational energy penalties were calculated by using the MacroModel module in Maestro and the energy penalties indicate the difference of the ligand potential energies between tightly restrained minimization and unrestrained minimization of the docking pose in water. The binding energies of the docking pose were calculated with the default parameters in Prime MMGBSA 3.0 version.

**Kinase Panel Protocol.** Activity-based kinase profiling was performed by either radioactivity assays using [γ-33P] ATP or AlphaScreen assays (PerkinElmer) with anti-phosphotyrosine antibody. The kinase profiling was performed by KINOMEScan Profiling Service of DiscoverX corporation. Compound 13 was screened at 0.3 μM, and assay results are described as % Ctrl. 

**In Vitro Inhibitory Activity against ALK and TrkA Kinase.**

**Protein Preparation.** The expression plasmid for human ALK kinase domain (residues 1058–1620, Genebank Accession no. NM_004304) was constructed in a pFastBacGST vector (Thermo Fisher Scientific). Recombinant proteins of ALK were prepared using a baculovirus expression system. The GST-tagged TrkA kinase domain (residues 436–790, Genebank accession no. NM_001012331.1) was purchased from Carna Biosciences (Kobe, Japan).

**Cell-Based ALK Autophosphorylation Assay.** The full-length cDNA encoding human ALK (NM_004304) construct was amplified by PCR and cloned into the pcDNA3.3 vector (Thermo Fisher Scientific). Human embryonic kidney 293 (HEK293) cell line was cultured with Dulbecco’s modified Eagle’s medium (Thermo Fisher Scientific) containing 10% fetal bovine serum (FBS), 100 U/mL of penicillin-G, and 100 μg/mL of streptomycin sulfate (Thermo Fisher Scientific). For evaluation of compound inhibitory activity in cells, the suspended HEK293 cells were transfected with expression plasmids encoding the ALK full-length construct using the Fugene HD reagent (Roche Diagnostics, Basel, Switzerland) according to the manufacturer’s instructions. The cell suspension was seeded into 96-well culture plates at 3.0 × 10^4 cells/well using Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% FBS and incubated two overnights in a 5% CO_2_ atmosphere at 37 °C. After incubation for two overnights, cells were treated with various concentrations of test compounds for 60 min in 10% FBS containing DMEM. The cells were then lysed by adding cell lysis buffer (Cell Signaling Technology, Danvers, MA). Quantification of phopho-ALK (Tyr1604) was performed by using Phospho-ALK (Tyr1604) Sandwich ELISA Kit (Cell Signaling Technology) according to the manufacturer’s instructions. Percentage inhibition was calculated from the signal intensity using the following formula

\[
\% \text{ inhibition} = 100 - \left( \frac{A - X}{A - B} \right) \times 100
\]

A: compound nontreatment, B: positive control (10 μM NVP-TAE684) treatment, and X: test compound.

The IC_{50} values and 95% CIs were estimated using a four-parameter logistic curve using XLfit software (IDBS, London, UK).

**Molecular Biology, Protein Purification, and Crystallization of human ALK.** A clone corresponding to the kinase catalytic domain region of human ALK kinase residues 1090 to 1406 was cloned into the pFastBacHTb vector (Thermo Fisher Inc.). It contained an N-terminal 6X Histidine tag with a cleavable TEV protease recognition site and a polyhedron replication origin site. It was subcultured in the Baculovirus insect cell strain SF9 (Spodoptera frugiperda, Thermo Fisher Inc.) for large-scale expression and purification. Cells were lysed by sonication, followed by centrifugation at 14,000 rpm for 1 h. The supernatant was loaded on a nickel-charged NTA column and purified by linear gradient of increasing nickel sulfate (20–200 mM). The eluate was checked for purity and treated with TEV protease. The final step was size exclusion chromatography on a Superdex 75 column. After confirmation of the purity of the fractions and their monodispersity, protein was concentrated to 25 mg/mL in buffer containing 20 mM Tris pH 8.0, 150 mM NaCl, 0.5 mM EDTA, 1 mM benzamidine, and 0.25 mM TCEP and used for structural studies. Initial screening in the Hampton PEG Ion screen yielded several hits both with apo protein and in the presence of 1 mM concentration of compounds. Best diffracting crystals appeared when optimized under the following conditions: 19–25% PEG 3350, 2.0% Tascimate pH 7.0, and a 7.3 or 8.2 ratio of Tris chloride: Tris sodium (100 mM final concentration). Crystals were harvested after cryoprotection with either 15% ethylene glycol or 15% glycerol for 15 min.

**Crystal Structure Determination.** Data was collected at beamline 5.0.3 of the Advanced Light Source at Berkeley, USA, and beamline X6A of the National Synchrotron Light Source at Brookhaven, USA. The structure was solved by molecular replacement using MOLREP, a part of the CCP4 package. The structure of ALK kinase (PDB ID 3L9P) was used as the
water molecules were fit manually after interpreting the difference map and accounting for the protein atoms. Crystallographic data collection and refinement statistics are summarized in the Supporting Information.

**ASSOCIATED CONTENT**

1. Supporting Information

The Supporting Information is available free of charge at [https://pubs.acs.org/doi/10.1021/acsomega.0c04900](https://pubs.acs.org/doi/10.1021/acsomega.0c04900).

Enzymatic kinase selectivity profile, KINOMEscan profile of 12, data collections and refinement statistics of crystallography, 1H NMR and 13C NMR charts of 1 and 12, determination of absolute configuration and experimental section for X-ray structure analysis of 4, and determination of absolute configuration and experimental section for X-ray structure analysis of 13 (PDF)

Molecular formula strings (CSV)

**Accession Codes**

The PDB entry codes for the crystal structures reported in this work are as follows: F-1, 7JYT; F-5, 7JY5; 1, 7YR; and 12, 7Y4. Authors will release the atomic coordinates and experimental data upon article publication.

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**Notes**

The authors declare no competing financial interest.

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**ABBREVIATIONS**

Ac acetyl
ALK anaplastic lymphoma kinase
ATP adenosine triphosphate
Bn benzyl
CNS central nervous system
cod cyclooctadiene
DBU 1,8-diazabicyclo[5.4.0]undec-7-ene
DIAD diisopropyl azodicarboxylate
DME 1,2-dimethoxyethane
DMSO dimethyl sulfoxide
FBDD fragment-based drug design
HAC heavy atom count
HPLC high-performance liquid chromatography
HTS high-throughput screening
LAH lithium aluminum hydride
LE ligand efficiency
LHMD5 lithium hexamethyldisilazide
LLE ligand lipophilicity efficiency
mp melting point
MS mass spectrometry
Ns nosyl
NSCLC non-small cell lung cancer
PDB protein data bank
rmsd root-mean-square deviation
SAR structure–activity relationship
SBDD structure-based drug design
TFA trifluoroacetic acid
THF tetrahydrofuran
THP tetrahydropropyl

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