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Novel 2,4-diaminopyrimidines bearing fused tricyclic ring moiety for anaplastic lymphoma kinase (ALK) inhibitor

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ABSTRACT

In this study, a series of novel 2,4-diaminopyrimidines bearing fused tricyclic ring moiety was described for ALK inhibitor. The pyrazole, imidazole, 1,2,4-triazole, piperazine and phenanthridine moieties were employed at the 2-position of pyrimidine. Among the compounds synthesized, **28**, **29**, **36**, and **42** showed promising anti-ALK activities in enzymatic- and cell-based assays. *In vivo* H3122 xenograft model study showed that compound **29** effectively suppressed ALK-driven tumor growth, similar to the extent of ceritinib, suggesting that it could be used for a novel ALK inhibitor development.

Anaplastic lymphoma kinase (ALK) is one of the receptor tyrosine kinases and has been implicated in a variety of tumors including anaplastic large-cell lymphomas (ALCL), diffuse large B-cell lymphoma (DLBCL), inflammatory myofibroblastic tumors (IMT), and solid tumors.[1–4] Since *ALK* gene is fused with various partner genes, such as *NMP*, *EML4*, and *KIF5* genes, the resulting fusion protein is constitutively activated, leading to a state of oncogenic addiction.[5] The blocking of constitutively activated ALK signal with small molecules can inhibit uncontrolled tumor proliferation, thus inducing rapid cell death.[6,7] Several drugs targeting ALK, such as crizotinib, ceritinib, and alectinib have been approved for the treatment of ALK-driven non-small cell lung cancer (NSCLC) patients. Brigatinib and lorlantinib are currently undergoing evaluation in clinical trials. Crizotinib, the first-in-class drug, was approved in 2011 for the treatment of ALK-positive NSCLC patients (Fig. 1). Although treatment of NSCLC patients with crizotinib displayed dramatic suppression of tumor growth, patients eventually suffered from rebounded tumor due to drug resistance predominantly by ALK mutations including L1196M, F1174L, C1156M, G1202R, etc.[8,9] In addition, crizotinib is not effective against brain metastases owing to its poor BBB penetration.[10] Thereby, many efforts to overcome crizotinib-resistant issues and poor brain penetration have been intensively pursued, leading to second-generation ALK inhibitors, such as ceritinib, alectinib, and brigatinib. Ceritinib

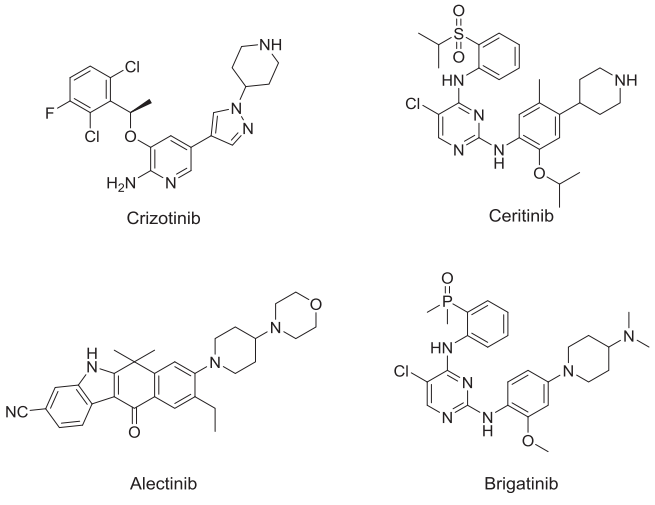

Fig. 1. ALK drugs approved.

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(LDK378) was approved for the treatment of ALK-positive cancer following treatment with crizotinib.[11,12] Ceritinib is effective against crizotinib-resistant mutants, L1196M, G1269A, and S1206Y, but failed to inhibit mutant G1202R and 1151Tins. As the recent data showed that brigatinib showed remarkable *in vitro* activity against both ALK wild type and mutants, it is considered a potential regimen for the patients with resistance to crizotinib, ceritinib, and alectinib.[13]

The ALK-targeted drug discovery program mostly focuses on the modification of substituents at the 2- and 4-positions of pyrimidines, as shown in ceritinib and brigatinib. In particular, the 2-position of the pyrimidines have been extensively studied to discover novel ALK inhibitors by introducing terminal secondary or tertiary amines that play an important role in interacting with Glu1210 of ALK.[14–16] Similarly, our efforts to develop novel ALK inhibitors have been reported, such as KRCA-0377 and 0713

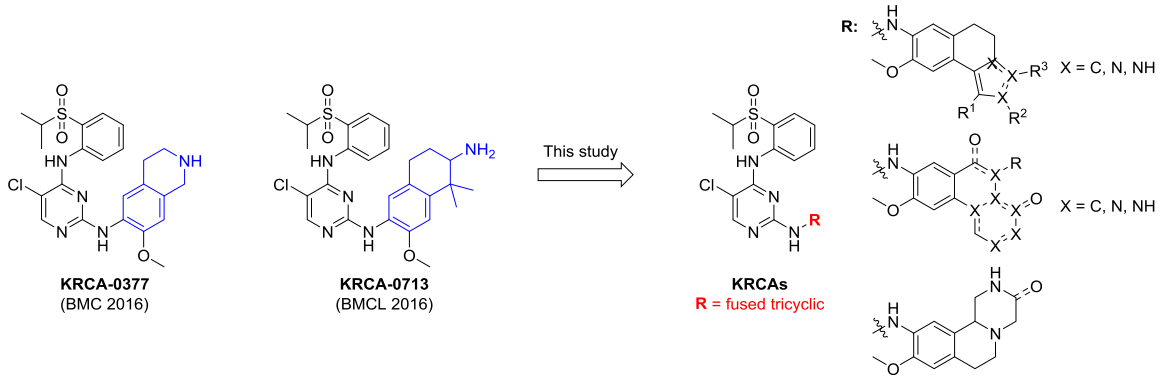
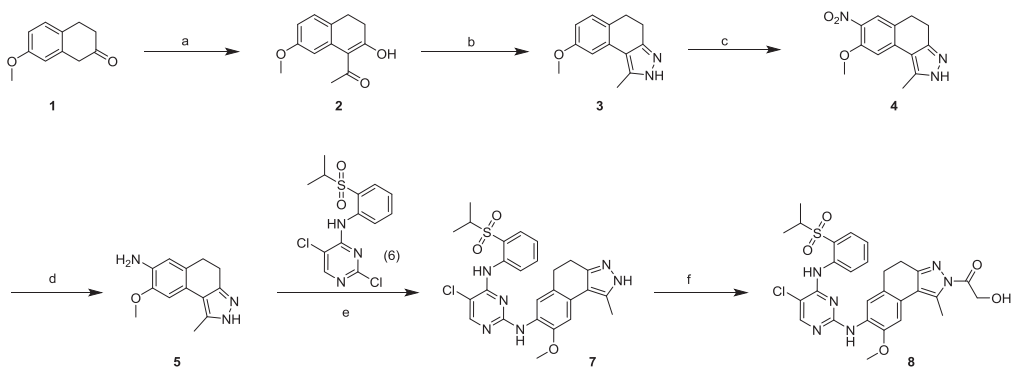
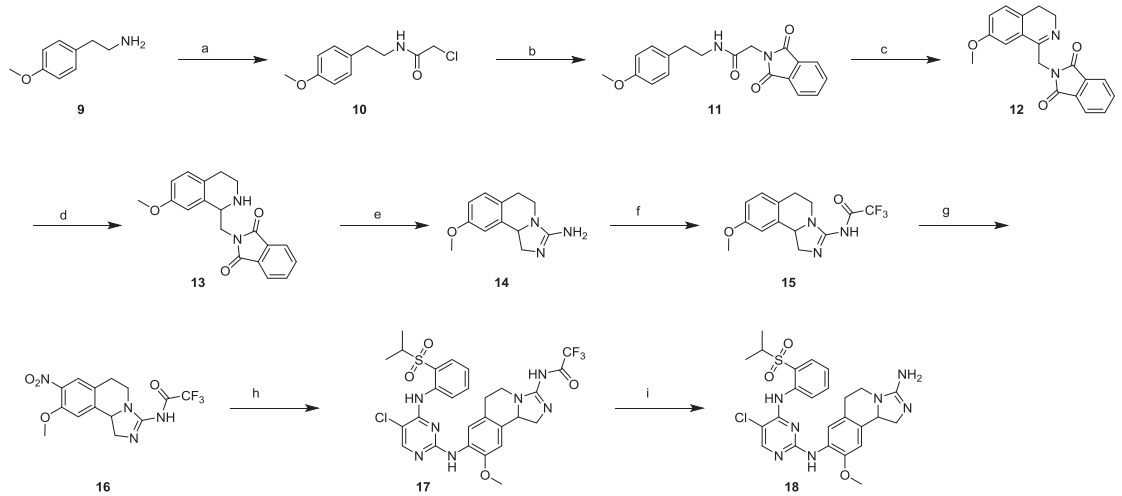


Fig. 2. Rational design of KRCAs in this study.



Scheme 1. Synthesis of compounds **7** and **8**. Reagents and conditions: (a) *N*-acetylimidazole, NaH, THF, 30%; (b) hydrazine monohydrate, EtOH, reflux, 92%; (c) HNO_3, TFA, rt, 69%; (d) H_2, 10% Pd/C, MeOH, rt, 18 h, 57%; (e) 0.08 M HCl in ethoxyethanol, 110 °C, 33%; (h) glycolic acid, EDCI, HOBt, TEA, DCM, rt, 67%.



Scheme 2. Synthesis of compound **18**. Reagents and conditions: (a) 2-chloroacetyl chloride, TEA, DCM, rt, 4 h, 76%; (b) potassium phthalimide, DMF, 90 °C, 12 h, 80%; (c) $POCl_3$, EtOH, 110 °C, 12 h, 60%; (d) cat. HCl, $NaBH_3CN$, MeOH, rt, 1 h, 99%; (e) hydrazine monohydrate, EtOH, 90 °C, 1 h, then CNBr, EtOH, rt, 1 h, 54%; (f) TFAA, TEA, DCM, rt, 1 h, 45%; (g) HNO_3, TFA, rt, 3 h, 55%; (h) i) H_2, 10% Pd/C, EtOAc, rt, 4 h; ii) **6**, 0.08 M HCl in ethoxyethanol, 80 °C, 12 h, 53% (two-step); (i) K_2CO_3, EtOH/H_2O, 90 °C, 1 h, 67%.

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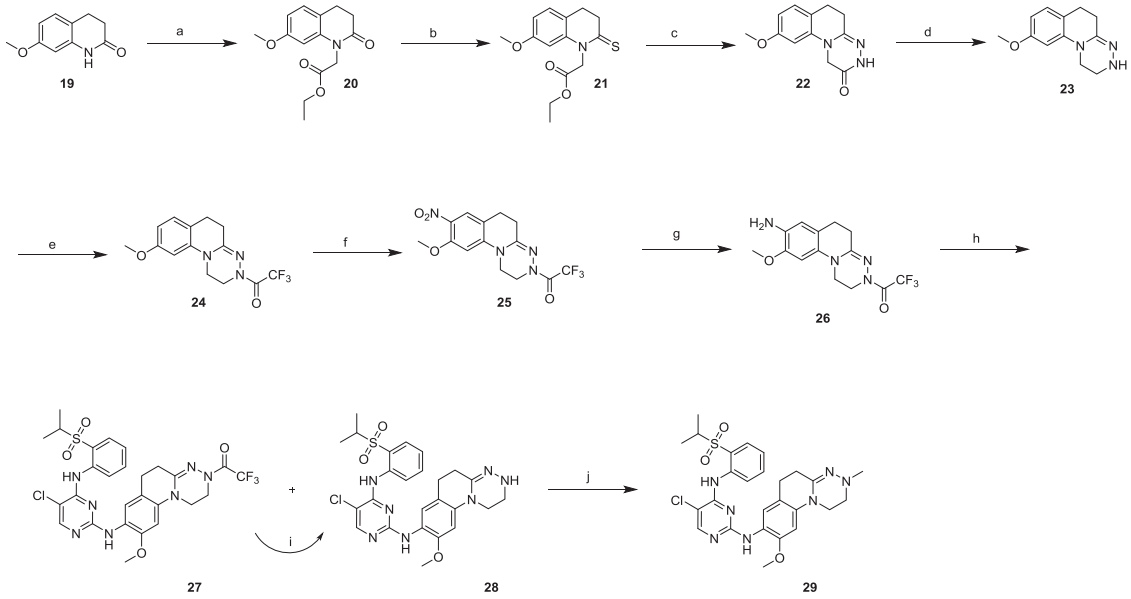
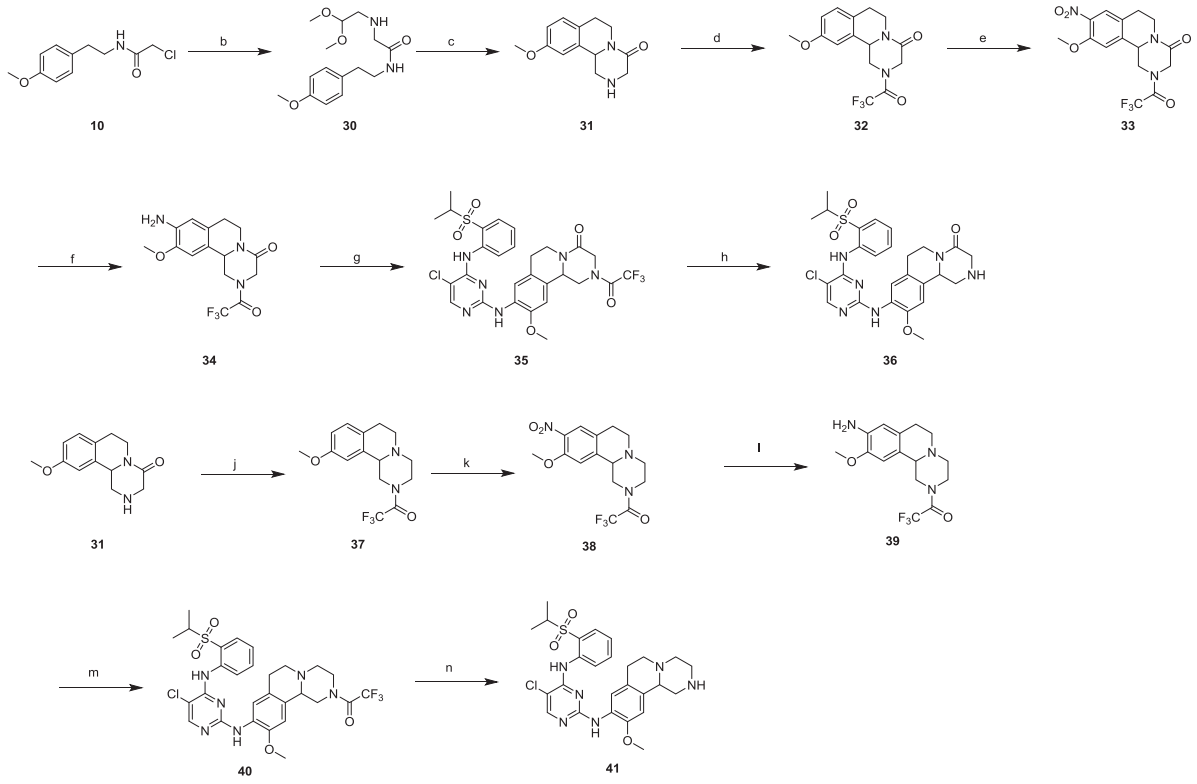
(Fig. 2), bearing bicyclic amines at the 2-position of the pyrimidine in ceritinib.[17–19]

During the course of our search for novel ALK inhibitors,[17–23] the encouraging biological activities of the bicyclic amines led us to further investigate tricyclic amines, since several precedent reports in other kinases, such as JAK3, PDGFR, and Pim, pointed out that tricyclic kinase inhibitors could improve activities as well as selectivities.[24–26] Thus, novel 2,4-diaminopyrimidine derivatives as ALK inhibitors were designed by replacing the 2-position substituent of pyrimidine in ceritinib with fused tricyclic scaffolds incorporating pyrazole, imidazole, piperazine, 1,2,4-triazine, and phenanthridine moieties (Fig. 2). Herein, we report the synthesis



Scheme 3. Synthesis of compounds **18** and **19**. Reagents and conditions: (a) Ethyl bromoacetate, NaH, DMF, rt, 79%; (b) Lawesson's reagent, toluene, reflux, 73%; (c) hydrazine monohydrate, EtOH, rt, 1 d, 84%; (d) LAH, THF, reflux, 3 h, 83%; (e) TFAA, TEA, DCM, rt, 64%; (f) HNO_3, TFA, rt, 47%; (g) H_2, 10% Pd/C, MeOH, rt, 18 h, 92%; (h) 0.08 M HCl in ethoxyethanol, 110 °C; (i) LiOH, MeOH/THF, H_2O, rt, 3 h, 68%; (j) formaldehyde, $NaBH_3CN$, AcOH (cat.), MeOH, rt, 24 h, 98%.



Scheme 4. Synthesis of **36** and **41**. Reagents and conditions: (a) $ClCOCH_2Cl$, TEA, DCM, rt, 4 h, 94%; (b) 2,2-diethoxyethan-1-amine, toluene, reflux, 2 h, 77%; (c) H_2SO_4, rt, 4 h, 28%; (d) TFAA, TEA, DCM, rt, 2 h, 72%; (e) HNO_3, TFA, 0 °C to rt, 2 h, 40%; (f) 10% Pd/C, H_2, EtOAc, rt, 12 h, 82%; (g) **6**, 0.08 M HCl in ethoxyethanol, 80 °C, 12 h, 43%; (h) LiOH·H_2O, THF/MeOH/H_2O, rt, 2 h, 40%; (j) i) $LiAlH_4$, THF, 50 °C, 2 h; ii) TFAA, TEA, DCM, rt, 2 h, 30%; (k) HNO_3, TFA, 0 °C to rt, 2 h, 40%; (l) 10% Pd/C, H_2, EtOAc, rt, 12 h, 89%; (m) **6**, 0.08 M HCl in ethoxyethanol, 80 °C, 12 h, 27%; (n) LiOH·H_2O, THF/MeOH/H_2O, rt, 2 h, 59%.

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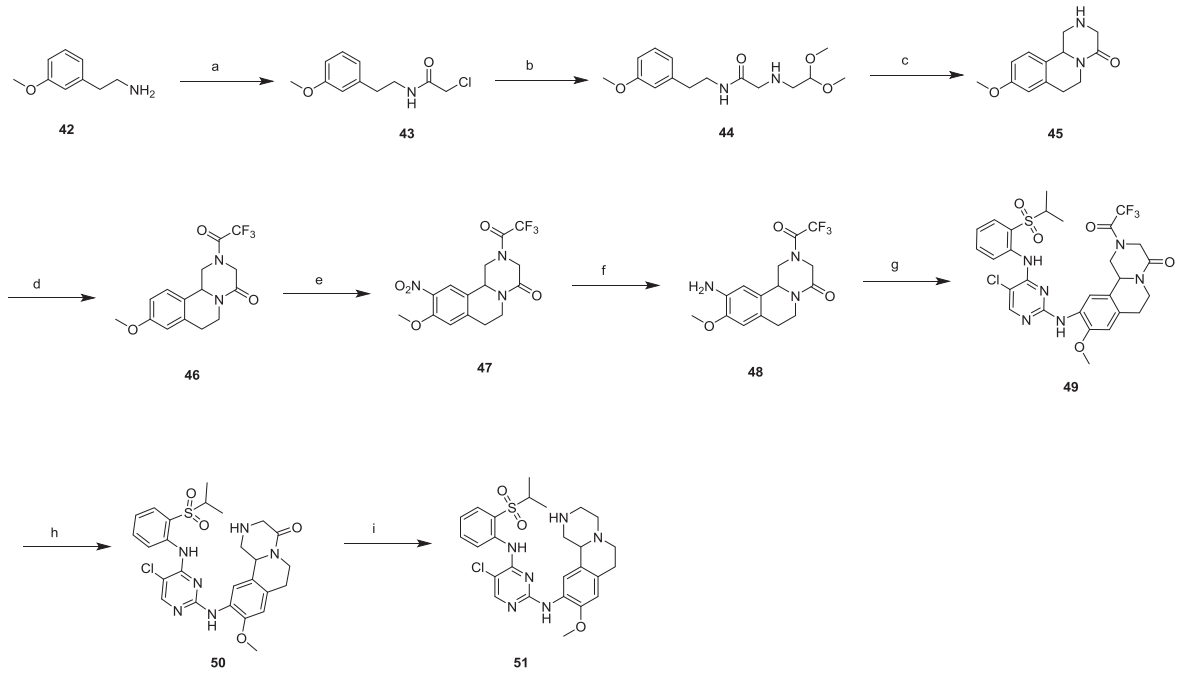
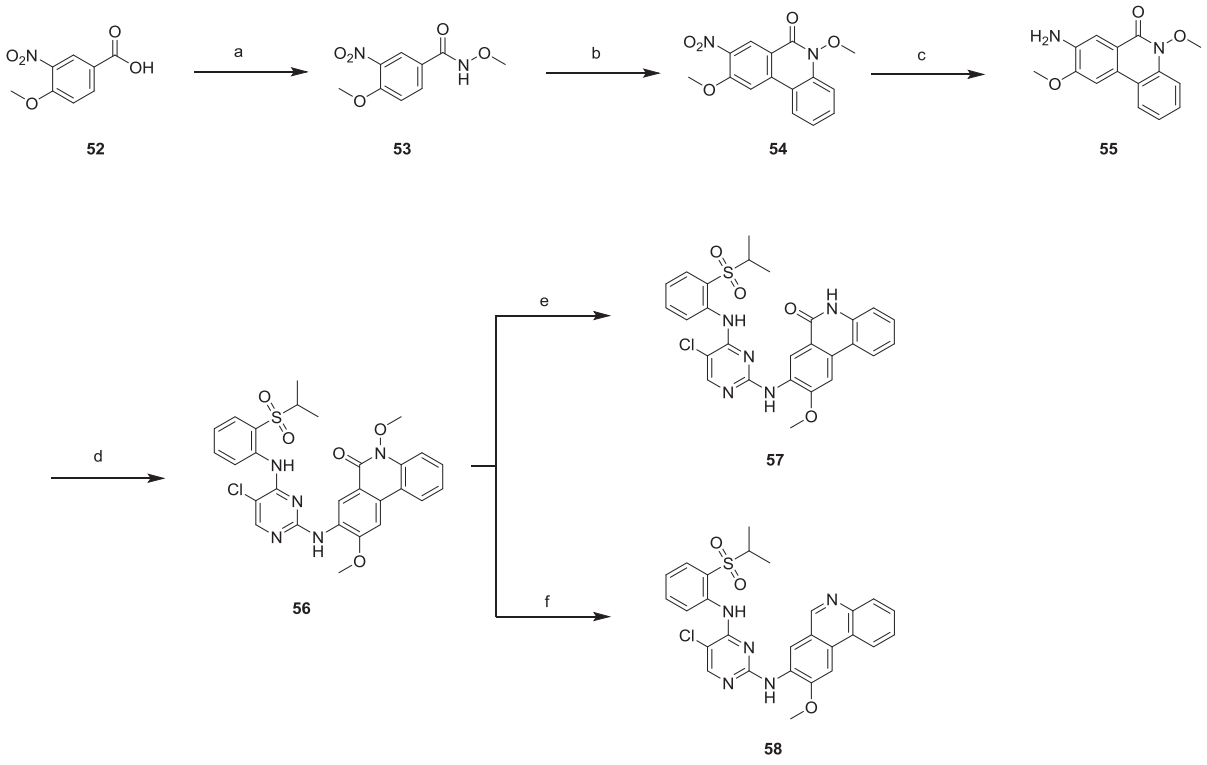
of designed compounds and their *in vitro* and *in vivo* biological evaluation results to provide more options for the discovery of ALK inhibitors.

Compounds **7** and **8** bearing pyrazole moiety were synthesized as shown in Scheme 1. 7-Methoxy-2-tetralone (**1**) was treated with *N*-acetylimidazole and NaH to afford compound **2**. Cyclization of **2**



Scheme 5. Synthesis of **50** and **51**. Reagents and conditions: (a) $ClCOCH_2Cl$, TEA, DCM, rt, 2 h, 93%; (b) 2,2-diethoxyethan-1-amine, toluene, reflux, 2 h, 82%; (c) CH_3SO_3H, DCM, rt, 12 h, 51%; (d) TFAA, TEA, DCM, rt, 2 h, 94%; (e) HNO_3, TFA, rt, 2 h, **59**%; (f) 10% Pd/C, H_2, MeOH, rt, 3 h, 80%; (g) **6**, 0.08 M HCl in ethoxyethanol, 80 °C, 12 h, 79%; (h) $LiOH{\cdot}H_2O$, THF/MeOH/H_2O, rt, 2 h, 80%; (j) $LiAlH_4$, THF, 50 °C, 2 h, 51%.



Scheme 6. Synthesis of compounds **56**, **57**, and **58**. Reagents and conditions: (a) i) oxalyl chloride, DMF (cat.), DCM, rt; ii) $MeONH_2{\cdot}HCl$, TEA, DCM, rt, 44%; (b) iodobenzene, Pd$(OAc)_2$, Ag_2O, AcOH, 120 °C, 36 h, 42%; (c) H_2, Pd/C, MeOH, rt, 3 h, 58%; (d) **6**, 0.08 M HCl in ethoxyethanol, 80 °C, 20 h; (e) LAH, THF, rt, 6 h, 63%; (f) i) $BH_3{\cdot}THF$, THF, 75 °C, 12 h; ii) 1.5 N HCl, 80 °C, 2 h, 40%.

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with hydrazine provided fused tricyclic pyrazole **3** in high yield.[27] Nitration of **3** gave nitro compound **4** as a major product. Reduction of nitro group in the presence of Pd/C afforded tricyclic pyrazoloaniline **5**. Compound **5** was coupled with commercially available 4-amino-2,5-dichlorpyrimidine **6** under acidic conditions to afford compound **7**. Compound **7** was treated with glycolic acid, EDCI, and HOBt to afford amide **8**.

In case of 2-aminodihydroimidazolo compound **18** (Scheme 2), at first 4-methoxyphenethylamine **9** was treated with 2-chloroacethyl chloride in the presence of TEA to afford amide **10**, which was transformed to phthalimidide **11** by treatment of potassium phthalimide for the Gabriel synthesis.[28] Intramolecular cyclization of **11** in the assistance of $POCl_3$ provided dihydroquinoline **12**, which was converted to tetrahydroisoquinoline **13** by the following $NaBHCN_3$ reduction. Hydrolysis of **13** using hydrazine followed by cyclization in the presence of CNBr gave 2-aminodihydroimidazole **14**.[29] With amine **14** in hand, sequential amine protection, nitration, nitro reduction, and coupling with **6** afforded **15**, **16**, **17**, and **18**, respectively.[19]

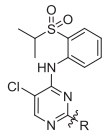
Compounds **28** and **29** bearing 1,2,4-triazine moiety were synthesized as described in Scheme 3. 7-Methoxy-3,4-dihydroquinolin-2-one (**19**) was treated with ethyl bromoacetate and NaH to afford *N*-alkyl compound **20**. The amide of **20** was converted to thioamide **21** by treatment of Lawesson's reagent. Cyclization of **21** with hydrazine provided fused tricyclic 1,2,4-triazinone **22**. Reduction of amide group in **22** by treatment of LAH provided dihydro-1,2,4-triazine **23**, which was treated with TFAA to afford **24**. Typical nitration, reduction, and coupling with **6** provided compounds **25**, **26**, and a mixture of **27** and **28**, respectively. Compound **27** was hydrolyzed by LiOH to afford **28**. Compound **28** was treated with formaldehyde in the presence of $NaBH_3CN$ and a catalytic amount of AcOH to facilitate compound **29**.

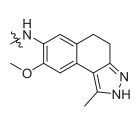
Piperazinoisoquinolines **36** and **41** were synthesized as shown in Scheme 4. Piperazinodihydroisoquinoline **31** was prepared from acid-mediated intramolecular cyclization of aminodimethylacetal **30**, which was generated from **10** through amination with 2,2-diethoxyethan-1-amine. With **31** in hand, sequential protection, nitration, nitro reduction, coupling with **6**, and deprotection delivered compounds **32**–**36**, respectively. For the preparation of tricyclic piperazine **41**, amide of oxopiperazine **31** was reduced by treatment of LAH and subsequent protection with TFAA afforded compound **37**. Tricyclic piperazine **41** was eventually obtained using compound **37** through similar reaction conditions and sequences for the preparation of **36**.

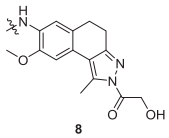
Compounds **50** and **51** were also prepared from the corresponding 3-methoxyphenethylamine **42** by similar synthetic manners as **36** and **41** (Scheme 5). Particularly, oxopiperazine **50** was reduced by LAH to afford tricyclic piperazine **51**.

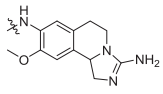
For phenanthridinones **56**–**57** and phenanthridine **58** (Scheme 6), the synthesis started from treatment of **52** with oxalyl

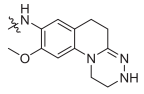
Table 1
ALK inhibitory activities of the final compounds.

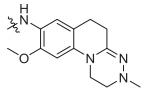


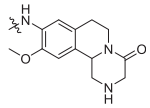


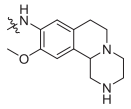


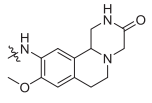


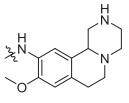


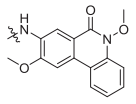


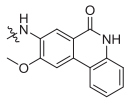


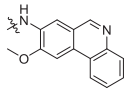












No.	ALK (wt.) IC_{50} (nM)	L1196M IC_{50} (nM)	IR IC_{50} (nM)	H3122 CC_{50} (nM)	BaF3 L1196M CC_{50} (nM)
Ceritinib	14	29	24	38	75
7	29	170	130	140	700
8	21	130	7.5	100	500
18	5.3	11	21	380	1300
28	0.33	8.3	11	38	260
29	1.7	8.1	6.0	36	120
36	3.7	5.2	2.8	30	93
41	2	1.9	2.1	36	75
50	4.3	5.3	2.2	770	310
51	12	4.8	14	240	790
56	190	380	300	160	2600
57	310	530	260	760	>10,000
58	>10,000	>10,000	>10,000	410	>10,000

The IC_{50} values are for the inhibition of ALK wild type and ALK L1196M mutant using homogenous time resolved fluorescence (HTRF) KinEASE-TK assay in a 384-well plate. The CC_{50} values are for cellular proliferation inhibition.

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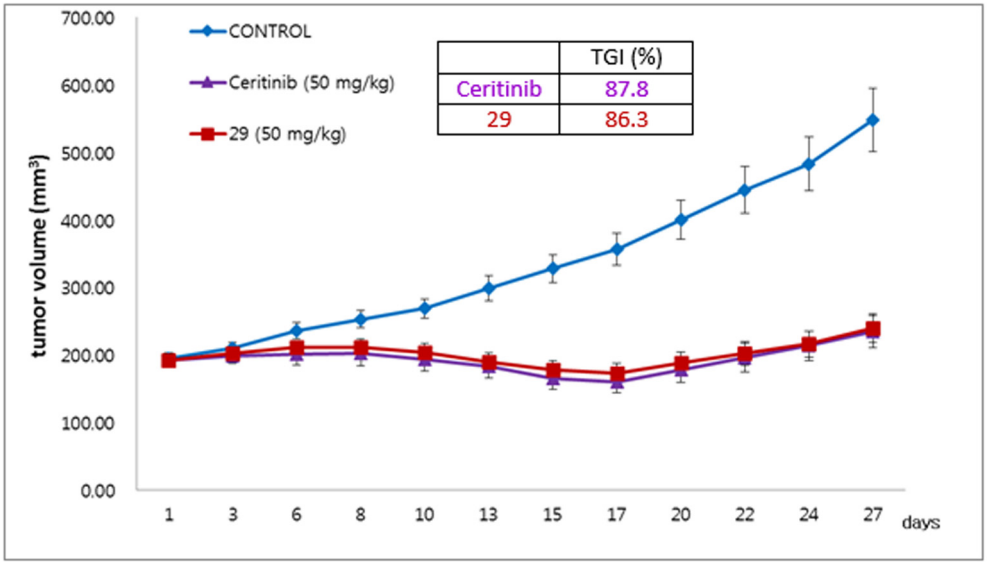

Fig. 3. Antitumor activity of ceritinib and **29** in H3122 xenograft model. Compounds were administered to SCID mice orally at a dose of 50 mg/kg q.d. for 14 days after the tumor volume reached 200 mm.3 Each group consisted of 8 mice. The results are shown as the mean ± standard error.

chloride and catalytic DMF followed by treatment with methoxyamine hydrochloride in the presence of TEA to afford amide **53**. Compound **53** was reacted with iodobenzene by the assistance of $Pd(OAc)_2$ and Ag_2O to give tricyclic *N*-methoxyphenanthridinone **54**.[30] Reduction of the nitro group in **54** and coupling reaction with **6** provided **55** and **56**, respectively. Reduction of **56** with LAH provided phenanthridine **57**, whereas reduction of **56** with BH_3 and HCl gave phenanthridine **58**

All final compounds synthesized were evaluated for their anti-ALK activities in biochemical assays (ALK wild type, L1196M, and insulin receptor kinase) and proliferation assays (H3122, BaF3 with L1196M) and summarized in Table 1. For kinase inhibition studies, homogenous time resolved fluorescence (HTRF) KinEASE-TK assays were performed in a 384-well plate. The proliferation assay was performed with H3122 (*wt.*) cells, which are EML4-ALK addicted non-small cell lung cancer cells. For ALK mutant cells, ALK L1196M gene was transfected in Ba/F3 cells. Pyrazoles (**7** and **8**), imidazole (**18**), 1,2,4-triazine (**28** and **29**), piperazine derivatives (**36**, **41**, **50**, and **51**) exhibited good ALK inhibitory activities with IC_{50} values in single- to low double-digit nanomolar ranges in wild type ALK enzymatic assays, while phenanthridine derivatives (**56**, **57**, and **58**) showed weak or no ALK inhibitory activities. In case of L1196M ALK mutant, this series of compounds displayed as similar activity trends as above. However, no drastic selectivity between ALK and IR was observed in this series. In cell based-assay, compound **28**, **29**, **36**, and **41** showed good anti-proliferative activities with IC_{50} values of 30–38 nM ranges in H3122 cells and this tendency was also observed in mutant cell lines (BAF3/L1196 M). It is worthy to note that compounds **36** and **41** showed better anti-ALK activities in cell-based assay than **50** and **51**, while these compounds are almost equal in enzymatic assays. Overall, four promising compounds (**28**, **29**, **36**, and **41**) displayed similar anti-ALK activities in comparison with ceritinib.

Among promising compounds in the cell-based assays, antitumor potency of **29** was examined using *in vivo* H3122 xenograft model. Compounds and controls (DMSO and ceritinib) were administered to SCID mice orally in 20% PEG400 and 3% Tween 80 in DDW at doses of 50 mg/kg q.d. for the 14-day duration of the study (Fig. 3). Tumor growth inhibition and regression were observed during drug treatment for 14 days. Although tumor regrowth was slowly observed after discontinuation of dose, compound **29** exhibited similar antitumor potency compared to ceritinib. No changes in body weight or side effects were observed during the study (data not shown).

In conclusion, the design, synthesis, and anti-ALK activity of novel 2,4-diaminopyrimidines bearing fused tricyclic ring moieties were described. The fused tricyclic structures incorporating pyrazole, imidazole, 1,2,4-triazole, piperazine, and phenanthridine were applied at the 2-position of pyrimidine in ceritinib. Compound **28**, **29**, **36**, and **41** showed promising activities in both enzymatic- and cell-based assays, while others were less active in cell-based assays. An *in vivo* efficacy study with compound **29** against H3122 tumor xenograft model in mice demonstrated highly potent inhibitory activity, similar to ceritinib, suggesting that it might be used for a novel ALK inhibitor development.

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2017.03.073.

References

1. Galkin AV, Melnick JS, Kim S, et al. *Proc Natl Acad Sci USA*. 2007;104:270–275.
2. Koivunen JP, Mermel C, Zejnullahu K, et al. *Clin Cancer Res*. 2008;14:4275–4283.
3. Minoo P, Wang HY. *Int J Clin Exp Pathol*. 2012;5:397–410.
4. Sasaki T, Rodig SJ, Chirieac LR, et al. *Eur J Cancer*. 2010;46:1773–1780.
5. Ren H, Tan Z-P, Zhu X, et al. *Cancer Res*. 2012;72:3312–3323.
6. Christensen JG, Zou HY, Arango ME, et al. *Mol Cancer Ther*. 2007;6:3314–3322.
7. Soda M, Choi YL, Enomoto M, et al. *Nature*. 2007;448:561–566.
8. Cui JJ, Tran-Dube M, Shen H, et al. *J Med Chem*. 2011;54:6342–6363.
9. Sakamoto H, Tsukaguchi T, Hiroshima S, et al. *Cancer Cell*. 2011;19:679–690.
10. Toyokawa G, Seto T, Takenoyama M, et al. *Cancer Metastasis Rev*. 2015;34:797–805.
11. Shaw AT, Kim DW, Mehra R, et al. *N Engl J Med*. 2014;370:1189–1197.
12. Friboulet L, Li N, Katayama R, et al. *Cancer Discov*. 2014;4:662–673.
13. Zhang S, Anjum R, Squillace R. *Clin Cancer Res*. 2016.

14. Liu Z, Ai J, Peng X, et al. *ACS Med Chem Lett*. 2014;5:304–308.
15. Liu Z, Yue X, Song Z, et al. *Eur J Med Chem*. 2014;86:438–448.
16. Song Z, Yang Y, Liu Z, et al. *J Med Chem*. 2015;58:197–211.
17. Kang GA, Lee M, Song D, et al. *Bioorg Med Chem Lett*. 2015;25:3992–3998.
18. Song D, Lee M, Park CH, et al. *Bioorg Med Chem Lett*. 2016;26:1720–1725.
19. Achary R, Yun JI, Park CM, et al. *Bioorg Med Chem*. 2016;24:207–219.
20. Park CH, Choe H, Jang IY, et al. *Bioorg Med Chem Lett*. 2013;23:6192–6196.
21. Lee HJ, Latif M, Choe H, et al. *Arch Pharm Res*. 2014;37:1130–1138.
22. Yun JI, Yang EH, Latif M, et al. *Arch Pharm Res*. 2014;37:873–881.
23. Mathi GR, Kang CH, Lee HK, et al. *Eur J Med Chem*. 2017;126:536–549.
24. Goedken ER, Argiriadi MA, Banach DL, et al. *J Biol Chem*. 2015;290:4573–4589.
25. Gazit A, Yee K, Uecker A, et al. *Bioorg Med Chem*. 2003;11:2007–2018.
26. Kiriazis A, Vahakoski RL, Santio NM, et al. *PLoS ONE*. 2013;8:e55409.
27. Mosher WA, Bechara IS. *J Heterocycl Chem*. 1970;7:843–846.
28. M-y Xi, J-m Jia, H-p Sun, et al. *J Med Chem*. 2013;56:7925–7938.
29. Maillard MC, Perlman ME, Amitay O, et al. *J Med Chem*. 1998;41:3048–3061.
30. Wang G-W, Yuan T-T, Li D-D. *Angew Chem*. 2011;123:1416–1419.