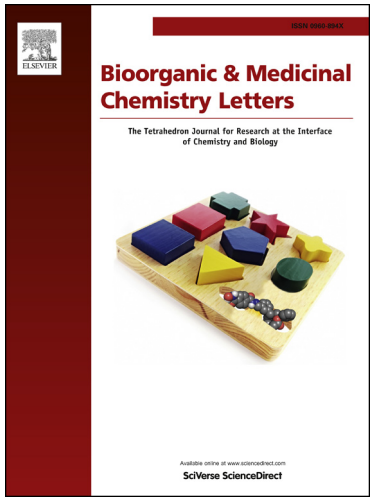

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Synthesis and evaluation of novel 2,4-diaminopyrimidines bearing bicyclic aminobenzazepines for anaplastic lymphoma kinase (ALK) inhibitor

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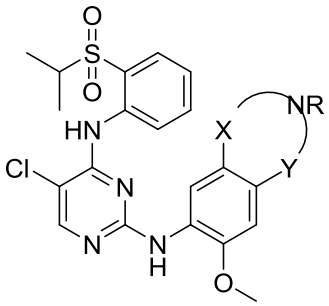
Abstract

A series of novel 2,4-diaminopyrimidine compounds bearing bicyclic aminobenzazepine were synthesized and evaluated for their anti-ALK activities. The activities of these compounds were confirmed in both enzyme- and cell-based ALK assays. Amongst compounds synthesized, KRCA-0445 showed very promising results in pharmacokinetic study and *in vivo* efficacy study with H3122 xenograft mouse model.



KRCAs

Keywords: Cancer, ALK, 2,4-diaminopyrimidine

Introduction

Anaplastic lymphoma kinase (ALK) belongs to the insulin receptor family of receptor tyrosine kinase (RTK). ALK-fused genes, such as NPM-ALK, EML4-ALK, KIF5-ALK, are putative oncogenes and responsible for many cancers including anaplastic large-cell lymphomas (ALCL), diffuse large B cell lymphoma (DLBCL), inflammatory myofibroblastic tumors (IMT), and a variety of solid tumors.[1-4] Crizotinib, Xalkori developed by Pfizer, is the first approved drug by FDA in 2011 for the treatment of ALK-positive lung cancer patients (Figure 1). Although crizotinib demonstrateed initial impressive efficacy, patients eventually developed resistance with ALK mutations including L1196M and C1156M.[5, 6] In 2014, LDK378, Ceritinib developed by Novartis, was approved for the treatment of ALK-positive cancer following treatment with crizotinib.[7, 8] LDK378 retains potency towards mutants, L1196M, G1269A, and S1206Y, but loses potency towards G1202R and 1151Tins. Alectinib having a benzo[*b*]carbazole moiety is also an ALK-selective second generation inhibitor developed by Roche. In terms of ALK selectivity, alcectinib is better than crizotinib.[6, 9] Alectinib is also active against the crizotinib-resistant ALK mutations L1196M, C1156Y, and F1174L in preclinical studies.[10] A Phase 3 clinical trial study of alectinib is currently ongoing for ALK-positive NSCLC

patients.[11], Others, AP26113 (Ariad Pharmaceuticals, Phase 2)[12], PF-06463922 (Pfizer, Phase 1/2),[13] and CEP-37440 (Teva, Phase 1),[14] are currently undergoing evaluation in clinical trials. Among those ALK inhibitors, LDK378 bearing a methyl group para to isopropoxy moiety was designed to circumvent the formation of glutathione adduct from NVP-TAE684, which was originally reported as a highly potent ALK inhibitor.[1, 15] The CEP-37440 contains a novel 1,4-diaminopyrimidine structure bearing distinguishing methylacetamidoaniline and bicyclic amine moieties, which are quite differentfrom LDK378. In our effort to discover potent ALK inhibitors,[16-18] we came up with hybridized structures of LDK378 and CEP-37440, thereby leading to a novel structure, KRCAs, which is 2,4-diaminopyrimidine bearing 4-(isopropylsulfonyl)anilino and bicyclic benzazepine moiety as shown in Figure 2. Although Liu et al. recently reported 2,4-diarylaminopyrimidine analog bearing bicyclic benzazepine moiety and methylacetamidoaniline as a c-Met/ALK multikinase inhibitor,[19] its chemical features differ from KRCAs. Herein, we report the synthesis of KRCAs and their anti-ALK activities in biochemical and cellular assays as well as PK and *in vivo* xenograft data of the selected compounds.

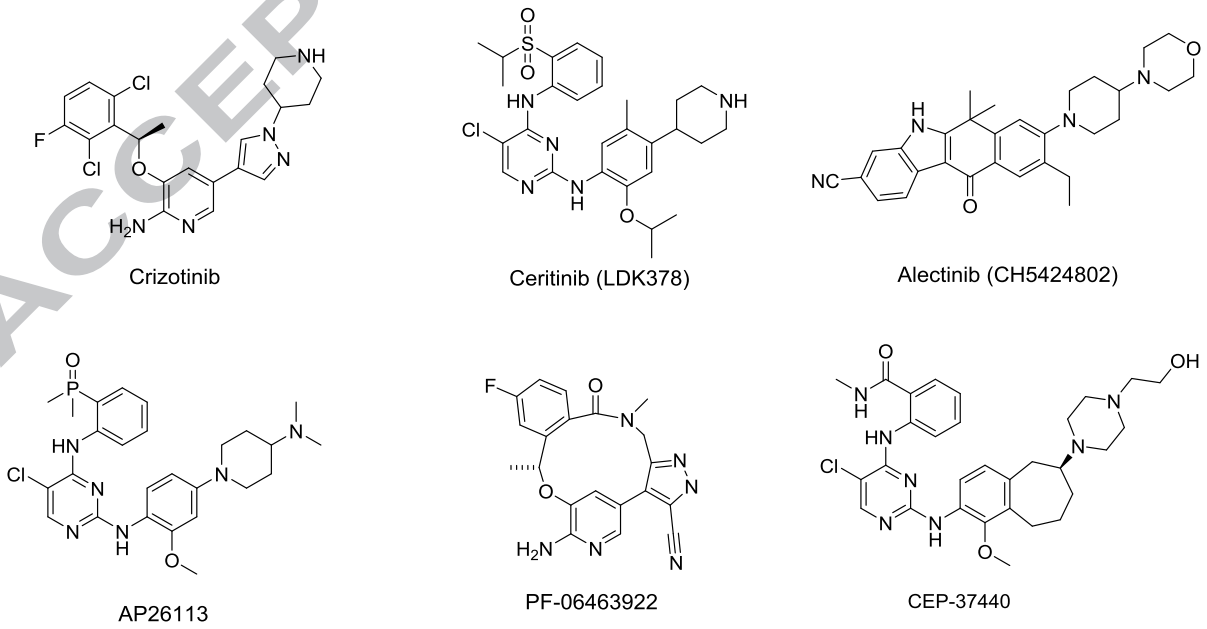


Figure 1. Structure of the potent ALK inhibitors.

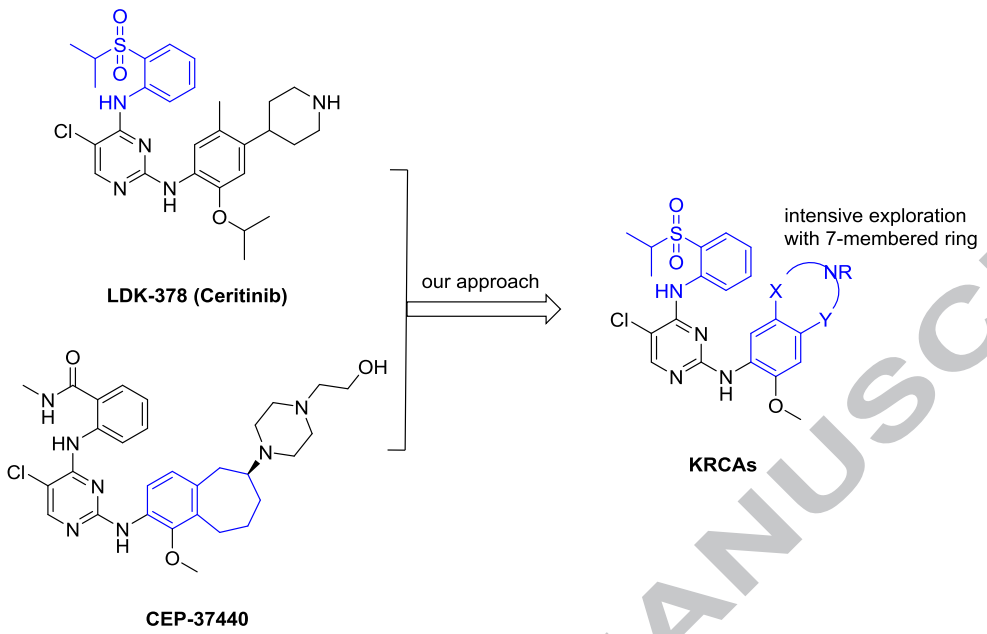
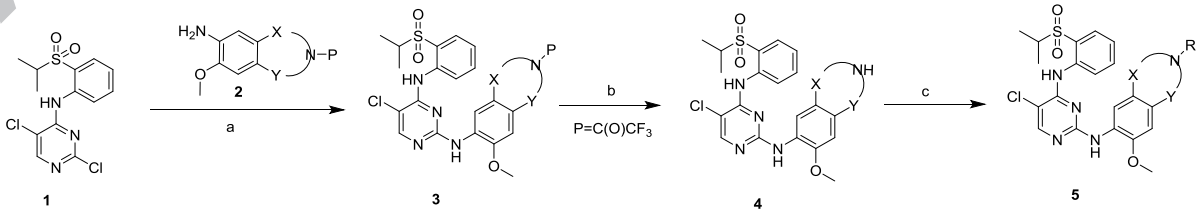


Figure 2. Rational design of KRCAs.

The representative synthesis of KRCA analogs is shown in Scheme 1. Commercially available 4-amino-2,5-dichloropyrimidine **1** was coupled with aminobenzazepines **2** under acidic conditions to afford compounds **3**,[20, 21] which were converted to the final compounds **4** by following hydrolysis of trifluoroacetamide group with K_2CO_3 in MeOH. Compounds **4** were reacted with various electrophiles such as alkyl halides, acids, and ketones for further diversification.

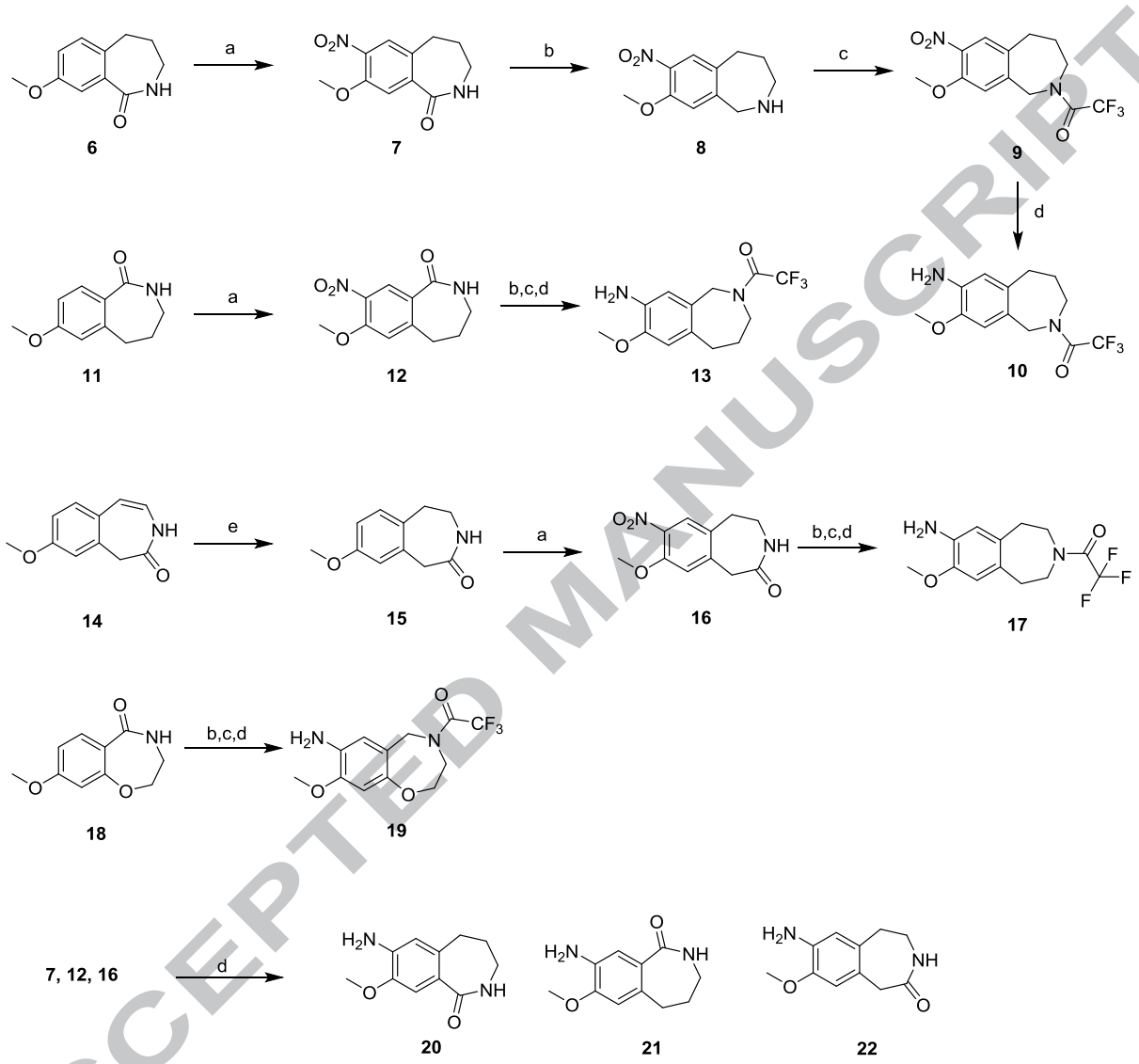
Scheme 1. General synthesis of KRCAs.



Reagents and conditions: (a) 4 *N* HCl in dioxane, 2-ethoxyethanol, 80 ℃, overnight; (b) K_2CO_3, MeOH, 90 ℃; (c) electrophiles (alkyl halides, acids, and ketones).

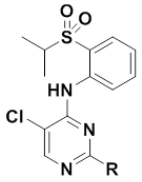
The bicyclic aminobenzazepines **2** were synthesized from the corresponding starting material as shown in Scheme 2. Nitrobenzazepinone **7** was prepared by nitration with KNO_3 from benzazepinone **6,** which was synthesized from the corresponding 1-tetralone by applying Schmidt reaction with NaN_3/HCl.[22] Reduction of amide group in compound **7** by treatment of BH_3/THF provided benzoazepine **8**, which was treated with trifluoroacetic anhydride (TFAA) to afford compound **9**. Reduction of nitro group in assistance of Pd/C gave the aminobenzazepine **10**. Compound **13** was prepared from intermediate **11** by same synthetic manner described above (nitration → amide reduction → protection → nitro reduction). Compound **17** was prepared from compound **14** which was synthesized by known procedure.[23] Compound **14** was hydrogenated over 10% Pd/C in AcOH to give amide **15**. A following series of reactions (nitration → amide reduction → protection → nitro reduction) provided compound **17**. Benzoxazepinone **18**, which was synthesized from resorcinol by known procedure in the literature,[24] was subjected to a series of reactions as described above to afford the benzoxazepine **19**. Reduction of nitro compounds **7**, **11**, and **15** gave aminobenzazepinones **20-22**, respectively.

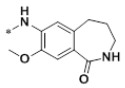
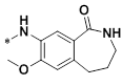
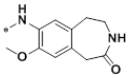
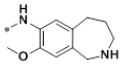
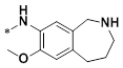
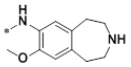
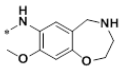
Scheme 2. Synthesis of aminobenzazepine analogs.



Reagents and conditions: (a) KNO_3, TFAA, MeCN, 0 ^oC to rt; (b) BH_3, THF, reflux; (c) TFAA, TEA, DCM, rt; (d) H_2, 10% Pd/C, MeOH, rt; (e) H_2, 10% Pd/C, AcOH, MeOH, rt.

Table 1. Activity profiles of compound **23-29**.



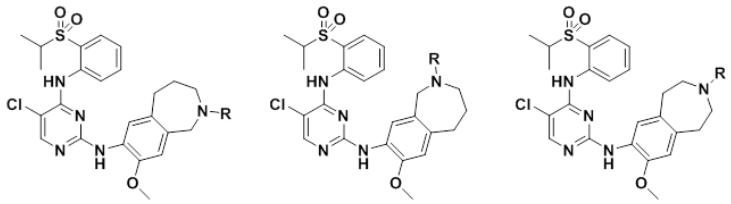
No.	Name	R	ALK *wt.* IC_{50} (nM)	ALK L1196M IC_{50} (nM)	IR IC_{50} (nM)	H3122 *wt.* CC_{50} (nM)	Ba/F3 L1196M CC_{50} (nM)
-	LDK378	-	14±4	29±10	93±35	38±7	75±9
23	KRCA-0504		2.7±0.5	3.0±0.2	25±14	121±21	106±21
24	KRCA-0463		4.4±1.9	12±7	21±12	216±36	774±528
25	KRCA-0465		5.2±2.7	15±4	11±3	111±16	201±59
26	KRCA-0444		1.7±0.2	2.7±0.3	5.2±3.4	44±11	15±3
27	KRCA-0462		1.6±0.2	3.4±1.2	5.1±2.1	50±38	31±6
28	KRCA-0445		1.8±0.4	1.6±0.7	2.0±0.7	37±6	41±14
29	KRCA-0503		1.5±0.7	5.8±0.9	12±3	32±4	25±8

The IC_{50} values are for the inhibition of ALK wild type, ALK L1196M mutant, and Insulin Receptor using homogenous time resolved fluorescence (HTRF) KinEASE-TK assay in 384-well plate. The CC_{50} values are for cellular proliferation inhibition. Data were fitted to a four-parameter sigmoidal dose response for determination of IC_{50} and CC_{50} values. The errors were reported as the 95% confidence interval.

All final compounds synthesized were evaluated for their anti-ALK activity in biochemical and cell-based assays. For kinase inhibition assay, ALK wild type (*wt*.), ALK L1196M, and insulin receptor (IR) homogeneous time resolved fluorescence (HTRF) KinEASE-TK assays were used in 384-well plate. The cell cytotoxicity assay was conducted with H3122 (*wt*.) cells which are EML4-ALK addicted non-small cell lung cancer cells. For ALK mutant cells, Ba/F3 cells transfected with ALK

L1196M were used. The KRCAs bearing various aminobenzazepine moieties were evaluated for anti-ALK potency as summarized in Table 1. While no selectivity against insulin receptor was observed, all compounds (**23-29**) exhibited very good ALK inhibitory activity with low nanomolar of IC_{50} values in both wild type and mutant ALK enzymatic assays. Most of compounds in this series displayed better enzymatic activities than those of LDK378. In cell-based assay with H3122 cells, benzazepines **26-29** bearing basic nitrogen atom showed low double-digit nanomolar of IC_{50} values, whereas benzazepinones **23-25** bearing relatively acidic amide group turned out to be less potent. Compounds **26-29** exhibited similar anti-proliferative activities against H3122 cells compared to LDK378 although those were at least 7-fold more active than LDK378 in ALK *wt.* enzymatic assay. These patterns were also observed in Ba/F3 L1196 mutant cells.

Table 2. Activity profiles of compounds **30-46**.



A B C

No.	Name	Structure	R	ALK *wt.* IC_{50} (μM)	ALK L1196M IC_{50} (μM)	H3122 *wt.* IC_{50} (μM)	Ba/F3 L1196M IC_{50} (μM)
30	KRCA-0541	A		0.39±0.09	1.4±0.2	68±22	67±16
31	KRCA-0506	B	Et	2.0±1.0	7.2±3.8	152±21	86±16
32	KRCA-0590	C		1.0±0.8	6.1±2.0	36±9	72±4
33	KRCA-0542	A		1.9±0.3	2.7±0.2	155±23	80±20
34	KRCA-0507	B		0.8±0.3	7.6±3.4	735±159	762±368
35	KRCA-0543	A		1.0±0.3	1.3±0.2	77±16	101±25
36	KRCA-0508	B		4.5±2.1	34±17	442±92	798±650
37	KRCA-0591	C		2.1±0.3	10±2	43±7	74±4
38	KRCA-0544	A		0.6±0.1	0.7±0.2	57±22	45±11
39	KRCA-0509	B		1.7±0.3	35±23	61±14	66±10
40	KRCA-0592	C		3.2±0.9	11±1	53±6	94±45
41	KRCA-0545	A		0.7±0.2	1.0±0.1	363±111	367±53
42	KRCA-0510	B		2.5±0.4	4.4±0.9	650±152	346±64
43	KRCA-0546	A		0.8±0.1	1.2±0.2	52±11	82±28
44	KRCA-0511	B		1.5±0.1	7.3±0.5	68±13	85±4
45	KRCA-0547	A		2.0±0.5	2.4±0.5	54±8	80±17
46	KRCA-0548	B		3.6±1.7	3.4±0.9	722±140	606±120

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 384-well plate. The CC_{50} values are for cellular proliferation inhibition. Data were fitted to a four-parameter sigmoidal dose response for determination of IC_{50} and CC_{50} values. The errors were reported as the 95% confidence interval.

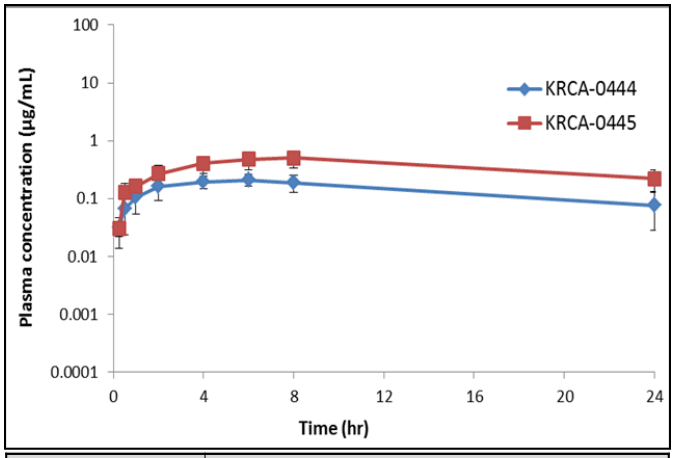
Next, several *N*-substituted benzazepine analogs (**30-46**) were evaluated and the data were summarized in Table 2. Interestingly, all compounds in this series exhibited very potent and similar

anti-ALK (*wt.*) activity ranges regardless of substituents. This trend was also observed in L1196M mutant enzyme assay, except for compounds **36** and **39**. However, substitution on nitrogen generally led to reduced anti-proliferative activity in both wild type (H3122) and mutant (Ba/F3, L1196M) cell-based assays. Overall, encouraging effect by recruiting substituents on nitrogen was not observed in this series.

Table 3. Metabolic stability of the selected compounds

Compound	Metabolic Stability Mouse / Human (% remaining at 30 min)
26 (KRCA-0462)	25 / 72
27 (KRCA-0444)	59 / 53
28 (KRCA-0445)	67 / 92
29 (KRCA-0503)	7 / 10

Amongst compounds tested in this study, four active compounds (**26-27**) in both enzymatic and cell-based assay were tested in human and mouse liver microsomal models to aid in prediction of metabolic stability (Table 3). Compounds **27** and **28** have reasonable stability in both human and mouse microsomal models but compound **26** has relatively short half-life. Compound **29** quickly metabolized in both mouse and human microsomal models. Based on this results, compounds **27** (KRCA-0444) and **28** (KRCA-0445) were selected for pharmacokinetic study. Serum concentrations were determined using LC-MS/MS after oral administration (PO) of compounds (10 mg/kg). Both compounds showed promising pharmacokinetic parameters as good drug candidates summarized in Figure 3. It is worthwhile to note that the selected compounds showed very long half-life ($T_{1/2}$) with at least 12 h, thus expecting long exposure time in *in vivo* study.



Parameters	KRCA-0444 (PO, 10 mg/kg)	KRCA-0445 (PO, 10 mg/kg)
T_{max} (hr)	4.67 ± 1.15	8 ± 0
C_{max} (μg/mL)	0.229 ± 0.0351	0.507 ± 0.164
$T_{1/2}$ (hr)	12.5 ± 5.86	12.1 ± 8.1
AUC_t (μg·hr/mL)	3.49 ± 1.01	8.7 ± 1.38
AUC_∞ (μg·hr/mL)	5.16 ± 2.58	12.9 ± 2.23
CL (L/kg/hr)	-	-
V_{ss} (L/kg)	-	-
F (%)	-	-

-Values are means of data from three animals.
-Vehicle: DMSO 5%, PEG400 50%, DW 55%.

Figure 3. Pharmacokinetic parameters of KRCA-0444 and KRCA-0445 in male rats.

Although both KRCA-0444 and KRCA-0445 exhibited very similar anti-ALK activity ranges, KRCA-0445 was selected for *in vivo* study due to slightly better pharmacokinetic parameters, compared with KRCA-0444. The *in vivo* study for the antitumor efficacy of compound KRCA-0445 was assessed in H3122 tumor xenograft model in SCID mouse. Compound KRCA-0445 and controls (DMSO and LDK378) 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 (Figure 4). Although complete tumor regression was not observed, compound KRCA-0445 showed similar tumor growth inhibition, compared with LDK378. No changes in body weight or side effects were observed during the study.

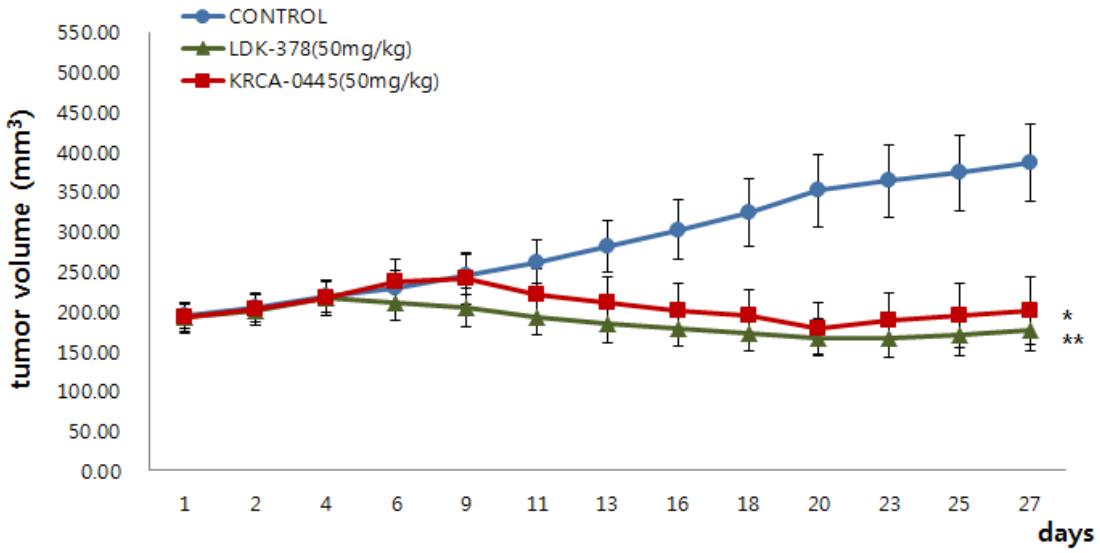


Figure 4. Antitumor activity of compound KRCA-0445 in H3122 xenograft model. Compounds were administered to SCID mice orally at doses of 50 mg/kg q.d. for 14-day after the tumor volumn reached around 200 mm^3. Each group consisted of 8 mice. The results were shown as the mean ± standard error. $^{**}p < 0.01$ and $^{*}p < 0.05$ vs control group on final day using Student's *t*-test.

Table 3. Kinase selectivity of KRCA-0445

Kinases	Inhibition at 100 nM (%)
ALK	100
FGFR1	97
ROS1	90
IGF-1R	78
EGFR (T790M, L858R)	73
c-Met	49
KDR	43
EGFR	31

The data was collected from Millipore's KinaseProfiler panel assays.

Kinase selectivity of KRCA-0445 was performed with several kinases as shown in Table 3. Interestingly, FGFR1, ROS1, IGF-1R, and EGFR (T790, L858R) were strongly inhibited by KRCA-

0445 at 100 nM concentration, while c-Met, KDR, and EGFR were slightly or weakly inhibited. Currently, many ALK inhibitors including crizotinib, ceritinib, and PF-06463922 are also under development for ROS1 inhibitor.[25-28] Therefore, KRCA-0445 could be further optimized to develop for potential ROS1 intervention. Moreover, it was reported that ceritinib inhibited both ALK and IGF-1R, thereby showing better potency in *in vivo* study, compared to crizotinib.[19] Likewise, KRCA-0445 targeting both ALK and IGF-1R might give clinical benefits in the future development.

In summary, the design and synthesis of novel 2,4-diaminopyrimidines bearing bicyclic aminobenzazepines and their anti-ALK activity were discussed. Amongst the compounds synthesized, compound **28** (KRCA-0445) showed promising assay results including anti-ALK activities and pharmacological properties. Kinase selectivity assay showed that KRCA-0445 is a multi-targeted inhibitor including FGFR1, ROS1 and IGF-1R, thus expecting synergy effect in anti-tumor activity. An *in vivo* efficacy study with compound KRCA-0445 demonstrated potent inhibitory activity against H3122 tumor xenograft model in mice, suggesting that it could be used as a platform for further optimization to develop novel ALK inhibitors.

Acknowledgments

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References and Notes

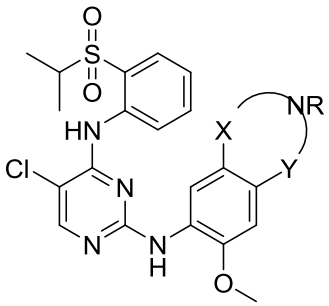
[1] A.V. Galkin, J.S. Melnick, S. Kim, T.L. Hood, N. Li, L. Li, G. Xia, R. Steensma, G. Chopiuk, J. Jiang, Y. Wan, P. Ding, Y. Liu, F. Sun, P.G. Schultz, N.S. Gray, M. Warmuth, Identification of NVP-TAE684, a potent, selective, and efficacious inhibitor of NPM-ALK, Proc Natl Acad Sci U S A, 104 (2007) 270-275.

[2] J.P. Koivunen, C. Mermel, K. Zejnullahu, C. Murphy, E. Lifshits, A.J. Holmes, H.G. Choi, J. Kim, D. Chiang, R. Thomas, J. Lee, W.G. Richards, D.J. Sugarbaker, C. Ducko, N. Lindeman, J.P. Marcoux, J.A. Engelman, N.S. Gray, C. Lee, M. Meyerson, P.A. Janne, EML4-ALK fusion gene and efficacy of an ALK kinase inhibitor in lung cancer, Clin Cancer Res, 14 (2008) 4275-4283.
[3] P. Minoo, H.Y. Wang, ALK-immunoreactive neoplasms, Int J Clin Exp Pathol, 5 (2012) 397-410.
[4] T. Sasaki, S.J. Rodig, L.R. Chirieac, P.A. Janne, The biology and treatment of EML4-ALK non-small cell lung cancer, Eur J Cancer, 46 (2010) 1773-1780.
[5] J.J. Cui, M. Tran-Dube, H. Shen, M. Nambu, P.P. Kung, M. Pairish, L. Jia, J. Meng, L. Funk, I. Botrous, M. McTigue, N. Grodsky, K. Ryan, E. Padrique, G. Alton, S. Timofeevski, S. Yamazaki, Q. Li, H. Zou, J. Christensen, B. Mroczkowski, S. Bender, R.S. Kania, M.P. Edwards, Structure based drug design of crizotinib (PF-02341066), a potent and selective dual inhibitor of mesenchymal-epithelial transition factor (c-MET) kinase and anaplastic lymphoma kinase (ALK), J Med Chem, 54 (2011) 6342-6363.
[6] H. Sakamoto, T. Tsukaguchi, S. Hiroshima, T. Kodama, T. Kobayashi, T.A. Fukami, N. Oikawa, T. Tsukuda, N. Ishii, Y. Aoki, CH5424802, a selective ALK inhibitor capable of blocking the resistant gatekeeper mutant, Cancer Cell, 19 (2011) 679-690.
[7] A.T. Shaw, D.W. Kim, R. Mehra, D.S. Tan, E. Felip, L.Q. Chow, D.R. Camidge, J. Vansteenkiste, S. Sharma, T. De Pas, G.J. Riely, B.J. Solomon, J. Wolf, M. Thomas, M. Schuler, G. Liu, A. Santoro, Y.Y. Lau, M. Goldwasser, A.L. Boral, J.A. Engelman, Ceritinib in ALK-rearranged non-small-cell lung cancer, N Engl J Med, 370 (2014) 1189-1197.
[8] L. Friboulet, N. Li, R. Katayama, C.C. Lee, J.F. Gainor, A.S. Crystal, P.Y. Michellys, M.M. Awad, N. Yanagitani, S. Kim, A.C. Pferdekamper, J. Li, S. Kasibhatla, F. Sun, X. Sun, S. Hua, P. McNamara, S. Mahmood, E.L. Lockerman, N. Fujita, M. Nishio, J.L. Harris, A.T. Shaw, J.A. Engelman, The ALK inhibitor ceritinib overcomes crizotinib resistance in non-small cell lung cancer, Cancer Discov, 4 (2014) 662-673.
[9] K. Kinoshita, K. Asoh, N. Furuichi, T. Ito, H. Kawada, S. Hara, J. Ohwada, T. Miyagi, T. Kobayashi, K. Takanashi, T. Tsukaguchi, H. Sakamoto, T. Tsukuda, N. Oikawa, Design and synthesis of a highly selective, orally active and potent anaplastic lymphoma kinase inhibitor (CH5424802), Bioorg Med Chem, 20 (2012) 1271-1280.
[10] T. Seto, K. Kiura, M. Nishio, K. Nakagawa, M. Maemondo, A. Inoue, T. Hida, N. Yamamoto, H. Yoshioka, M. Harada, Y. Ohe, N. Nogami, K. Takeuchi, T. Shimada, T. Tanaka, T. Tamura, CH5424802 (RO5424802) for patients with ALK-rearranged advanced non-small-cell lung cancer (AF-001JP study): a single-arm, open-label, phase 1-2 study, Lancet Oncol, 14 (2013) 590-598.
[11] ALEX Study: A Randomized, Phase Ⅲ Study Comparing Alectinib With Crizotinib in Treatment-Naive Anaplastic Lymphoma Kinase (ALK)-Positive Advanced Non-Small Cell Lung Cancer (NSCLC) Patients; https://clinicaltrials.gov/ct2/results?term=alectinib&Search=Search.
[12] A Phase 2, Multicenter, Randomized Study of AP26113; https://clinicaltrials.gov/ct2/results?term=ap26113&Search=Search.
[13] A Study Of PF-06463922 An ALK/ROS1 Inhibitor In Patients With Advanced Non Small Cell

Lung Cancer With Specific Molecular Alterations; https://clinicaltrials.gov/ct2/results?term=PF-06463922&Search=Search.
[14] To Determine the Maximum Tolerated Dose of Oral CEP-37440 in Patients With Advanced or Metastatic Solid Tumors; https://clinicaltrials.gov/ct2/results?term=CEP-37440&Search=Search.
[15] T.H. Marsilje, W. Pei, B. Chen, W. Lu, T. Uno, Y. Jin, T. Jiang, S. Kim, N. Li, M. Warmuth, Y. Sarkisova, F. Sun, A. Steffy, A.C. Pferdekamper, A.G. Li, S.B. Joseph, Y. Kim, B. Liu, T. Tuntland, X. Cui, N.S. Gray, R. Steensma, Y. Wan, J. Jiang, G. Chopiuk, J. Li, W.P. Gordon, W. Richmond, K. Johnson, J. Chang, T. Groessl, Y.Q. He, A. Phimister, A. Aycinena, C.C. Lee, B. Bursulaya, D.S. Karanewsky, H.M. Seidel, J.L. Harris, P.Y. Michellys, Synthesis, structure-activity relationships, and in vivo efficacy of the novel potent and selective anaplastic lymphoma kinase (ALK) inhibitor 5-chloro-N2-(2-isopropoxy-5-methyl-4-(piperidin-4-yl)phenyl)-N4-(2-(isopropylsulf onyl)phenyl)pyrimidine-2,4-diamine (LDK378) currently in phase 1 and phase 2 clinical trials, J Med Chem, 56 (2013) 5675-5690.
[16] H.J. Lee, M. Latif, H. Choe, I. Ali, H.K. Lee, E.H. Yang, J.I. Yun, C.H. Chae, J.K. Jung, H.R. Kim, C.O. Lee, C.H. Park, K. Lee, ALK inhibitors of bis-ortho-alkoxy-para-piperazinesubstituted-pyrimidines and -triazines for cancer treatment, Arch Pharm Res, 37 (2014) 1130-1138.
[17] C.H. Park, H. Choe, I.Y. Jang, S.Y. Kwon, M. Latif, H.K. Lee, H.J. Lee, E.H. Yang, J.I. Yun, C.H. Chae, S.Y. Cho, S.U. Choi, J.D. Ha, H. Jung, H.R. Kim, P. Kim, C.O. Lee, C.S. Yun, K. Lee, Novel bis-ortho-alkoxy-para-piperazinesubstituted-2,4-dianilinopyrimidines (KRCA-0008) as potent and selective ALK inhibitors for anticancer treatment, Bioorg Med Chem Lett, 23 (2013) 6192-6196.
[18] J.I. Yun, E.H. Yang, M. Latif, H.J. Lee, K. Lee, C.S. Yun, C.H. Park, C.O. Lee, C.H. Chae, S.Y. Cho, H.J. Jung, P. Kim, S.U. Choi, H.R. Kim, Novel 2,4-dianilino-5-fluoropyrimidine derivatives possessing ALK inhibitory activities, Arch Pharm Res, 37 (2014) 873-881.
[19] Z. Liu, J. Ai, X. Peng, Z. Song, K. Wu, J. Zhang, Q. Yao, Y. Chen, Y. Ji, Y. Yang, M. Geng, A. Zhang, Novel 2,4-Diarylaminopyrimidine Analogues (DAAPalogues) Showing Potent c-Met/ALK Multikinase Inhibitory Activities, ACS Med Chem Lett, 5 (2014) 304-308.
[20] D.E. Gingrich, J.G. Lisko, M.A. Curry, M. Cheng, M. Quail, L. Lu, W. Wan, M.S. Albom, T.S. Angeles, L.D. Aimone, R.C. Haltiwanger, K. Wells-Knecht, G.R. Ott, A.K. Ghose, M.A. Ator, B. Ruggeri, B.D. Dorsey, Discovery of an orally efficacious inhibitor of anaplastic lymphoma kinase, J Med Chem, 55 (2012) 4580-4593.
[21] G.R. Ott, R. Tripathy, M. Cheng, R. McHugh, A.V. Anzalone, T.L. Underiner, M.A. Curry, M.R. Quail, L. Lu, W. Wan, T.S. Angeles, M.S. Albom, L.D. Aimone, M.A. Ator, B.A. Ruggeri, B.D. Dorsey, Discovery of a potent inhibitor of anaplastic lymphoma kinase with in vivo antitumor activity, ACS Med Chem Lett, 1 (2010) 493-498.
[22] H. Ueno, K. Yokota, J. Hoshi, K. Yasue, M. Hayashi, Y. Hase, I. Uchida, K. Aisaka, S. Katoh, H. Cho, Synthesis and structure-activity relationships of novel selective factor Xa inhibitors with a tetrahydroisoquinoline ring, J Med Chem, 48 (2005) 3586-3604.
[23] M. Wang, M. Gao, B.L. Steele, B.E. Glick-Wilson, C. Brown-Proctor, A. Shekhar, G.D. Hutchins, Q.H. Zheng, A new facile synthetic route to [11C]GSK189254, a selective PET radioligand for

imaging of CNS histamine H3 receptor, Bioorg Med Chem Lett, 22 (2012) 4713-4718.
[24] C.T. Yen, K. Nakagawa-Goto, T.L. Hwang, P.C. Wu, S.L. Morris-Natschke, W.C. Lai, K.F. Bastow, F.R. Chang, Y.C. Wu, K.H. Lee, Antitumor agents. 271: total synthesis and evaluation of brazilein and analogs as anti-inflammatory and cytotoxic agents, Bioorg Med Chem Lett, 20 (2010) 1037-1039.
[25] website : https://clinicaltrials.gov/ct2/results?term=ROS1&Search=Search.
[26] H.Y. Zou, Q. Li, L.D. Engstrom, M. West, V. Appleman, K.A. Wong, M. McTigue, Y.L. Deng, W. Liu, A. Brooun, S. Timofeevski, S.R. McDonnell, P. Jiang, M.D. Falk, P.B. Lappin, T. Affolter, T. Nichols, W. Hu, J. Lam, T.W. Johnson, T. Smeal, A. Charest, V.R. Fantin, PF-06463922 is a potent and selective next-generation ROS1/ALK inhibitor capable of blocking crizotinib-resistant ROS1 mutations, Proc Natl Acad Sci U S A, 112 (2015) 3493-3498.
[27] A.T. Shaw, S.H. Ou, Y.J. Bang, D.R. Camidge, B.J. Solomon, R. Salgia, G.J. Riely, M. Varella-Garcia, G.I. Shapiro, D.B. Costa, R.C. Doebele, L.P. Le, Z. Zheng, W. Tan, P. Stephenson, S.M. Shreeve, L.M. Tye, J.G. Christensen, K.D. Wilner, J.W. Clark, A.J. Iafrate, Crizotinib in ROS1-rearranged non-small-cell lung cancer, N Engl J Med, 371 (2014) 1963-1971.
[28] A.T. Shaw, B.J. Solomon, Crizotinib in ROS1-rearranged non-small-cell lung cancer, N Engl J Med, 372 (2015) 683-684.

Graphical abstract



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