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Novel 2,3,4,5-tetrahydro-benzo[*d*]azepine derivatives of 2,4-diaminopyrimidine, selective and orally bioavailable ALK inhibitors with antitumor efficacy in ALCL mouse models

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ABSTRACT

The synthesis and biological evaluation of potent and selective anaplastic lymphoma kinase (ALK) inhibitors from a novel class of 2,4-diaminopyrimidines, incorporating 2,3,4,5-tetrahydro-benzo[*d*]azepine fragments, is described. An orally bioavailable analogue (**18**) that displayed antitumor efficacy in ALCL xenograft models in mice was identified and extensively profiled.

Anaplastic Lymphoma Kinase (ALK) is a member of the insulin receptor tyrosine kinase family. ALK was initially identified in a chimeric protein (NPM–ALK) arising from a chromosomal translocation between the *ALK* and nucleophosmin (*NPM*) genes.[1] NPM–ALK is constitutively active and plays an oncogenic role in 70–80% of all anaplastic large cell lymphomas (ALCL).[2] Two other ALK fusion mutants of relevance in cancer pathogenesis were recently described, tropomyosin 3 gene (TPM3–ALK) in inflammatory myofibroblastic tumors (IMT)[3] and echinoderm microtubule-associated protein-like 4 gene (EML4–ALK)[4] in non-small cell lung cancers (NSCLC). Overexpression of ALK was also observed in glioblastoma[5] and neuroblastoma.[6] Consequently, a number of ALK inhibitors in various stages of development as therapies for ALK-dependent cancers have been reported (Fig. 1), including PF-2341066 (**1**),[7] NVP-TAE684 (**2**),[8] and GSK1838705A (**3**).[9]

Recent efforts from our laboratories[10] towards ALK inhibitors with therapeutic utility led to the discovery of a novel and potent bridged bicyclic derivative (**4**, Fig. 1).[2b,11] During the course of these endeavors, we synthesized and profiled a variety of analogs related to the reported kinase inhibitor NVP-TAE226[12] (Table 1, **5**), which led inadvertently to **6** (Table 1), presumably arising from bis-addition of the requisite aniline to unreacted 2,4,5-trichloropyrimidine.

Interestingly, **6** demonstrated equivalent inhibition of ALK kinase activity to **5**, albeit with slightly lower (2.4-fold) cellular

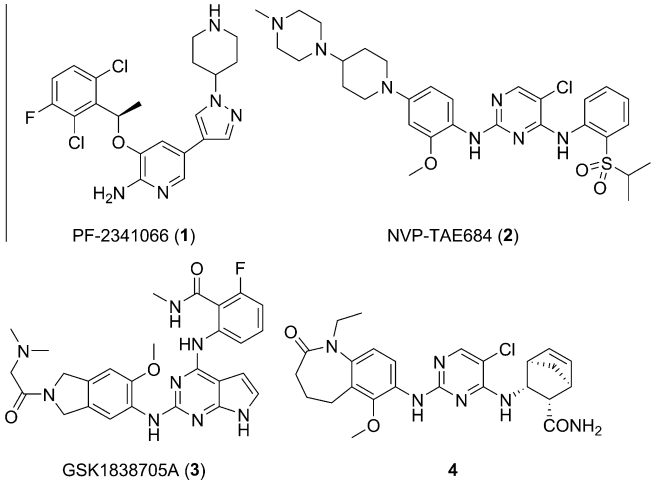

Figure 1. Examples of reported ALK inhibitors.

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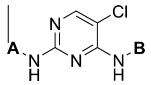
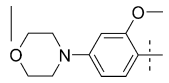
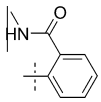
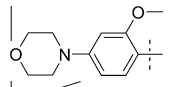
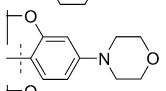
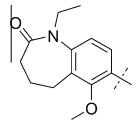
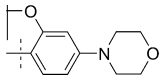
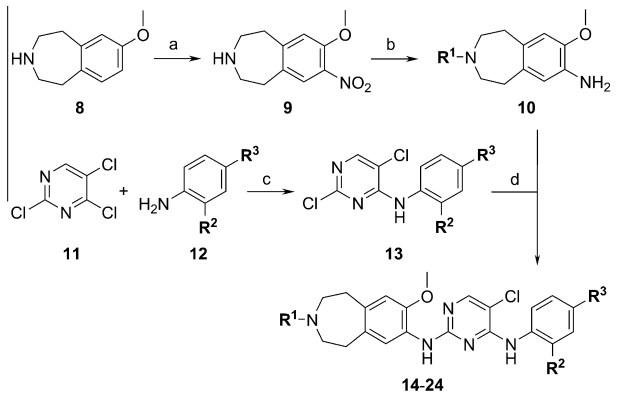
Table 1
Biological data for compounds **5**–**7**



Compds	A	B	IC_{50}[a] (nM)		
			ALK enzyme	ALK cell	IR enzyme
5			5	50	25
6			4	120	1860
7			34	175	>10,000

[a] IC_{50} values are reported as the average of at least two separate determinations.



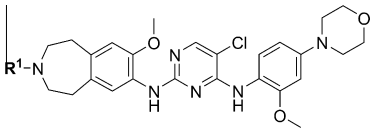
Scheme 1. Reagents and conditions: (a) (i) KNO_3, TFAA, MeCN, 0 °C→rt; (ii) separation of regioisomers; (iii) 20–40% aq NaOH, MeCN, rt; (b) (i) $\mathbf{R^1}$-X, K_2CO_3, MeCN, 75 °C; (ii) H_2, Pd/C, MeOH; (c) K_2CO_3, DMF or DIPEA, THF, rt to 50 °C; (d) HCl, $MeO(CH_2)_2OH$, 100 °C. See Tables 2 and 3 for $\mathbf{R^{1-3}}$ definitions.

activity. More importantly however, **6** exhibited a tremendous increase in selectivity against the highly homologous insulin receptor (IR). Selectivity over IR is desirable since recent reports of concomitant inhibition of IR with small molecule kinase inhibitors have shown compound-dependent fluctuation in glucose homeostasis in preclinical animal models.[9,13]

Capitalizing on this serendipitous finding, we incorporated morpholine-phenyl fragment **B** (Table 1) and benzazepinone **A** (cf. **4** in Fig. 1),[2b,11] providing novel compound **7**, though this resulted in a drop-off in both ALK enzymatic activity as well as cellular potency. IR selectivity was maintained. At this juncture we sought to improve the potency of these inhibitors while maintaining this high degree of IR selectivity. In an effort to provide synthetically tractable functionality with a handle to modulate both potency and physiochemical properties, the 2,3,4,5-tetrahydro-1*H*-benzo[*d*]azepine motif was incorporated into this pharmacophore.

The synthesis of these analogues is outlined in Scheme 1.[14] Benzazepine **8** was prepared from inexpensive starting materials employing reported procedures.[15] Nitration of **8** in presence of trifluoroacetic anhydride[16] afforded an intermediate trifluoroacetamide, which was hydrolyzed to the corresponding amine **9**. N-Alkylations followed by reduction of the nitro group generated anilines **10**. Reaction of 2,4,5-trichloropyrimidine (**11**) with anilines **12** secured intermediates **13**. Finally, a second S_NAr reaction between **10** and **13** provided the desired target molecules **14**–**24**.

Table 2
Biological data for compounds **14**–**18**



Compds	$\mathbf{R^1}$	IC_{50}[a] (nM)		
		ALK enzyme	ALK cell	IR enzyme
14	$MeO(CH_2)_2$–	7	150	2694
15	CHF_2CH_2–	58	—	>10,000
16	$MeS(O)_2CH_2CH_2$–	10	100	>3000
17	$MeNHC(O)CH_2$–	6	100	>3000
18	$(Me)_2NC(O)CH_2$–	4	30	1380

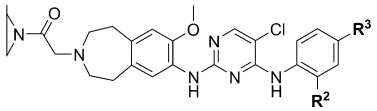
[a] IC_{50} values are reported as the average of at least two separate determinations.

Gratifyingly, enzyme activity of **14** (Table 2) was improved relative to **7** and comparable to **6** (Table 1). IR selectivity was maintained, though cellular activity still required improvement. We focused first on analogues diversely substituted on the benzazepine nitrogen and observed that subtle changes to the nature of $\mathbf{R^1}$ groups impacted both enzyme and cell activity.

Though likely not the only contributing factor, the basicity of this nitrogen appeared to be of particular importance. For example, fluorinated derivative **15** lost eightfold in enzyme activity relative to the more basic amine **14**. N-Substituents in **16** and **17** restored ALK activity for these compounds and brought moderate improvements in cellular activity. Amide **18** was the most promising lead (Table 2), active against ALK with good cell activity (cell/enzyme IC_{50} ratio of 7–8) and highly selective against IR (IR/ALK IC_{50} ratio of 345). These results prompted further exploration of the SAR with respect to the $\mathbf{R^{2,3}}$ substituents (Table 3).

Examples **19** and **20** comprise truncated versions of **18**, which define the selectivity determinants on the aromatic ring **B**. Compound **19** with a hydrogen as $\mathbf{R^2}$ resulted in a 12-fold loss in ALK activity relative to **18**. Furthermore, hydrogen in place of morpholine for $\mathbf{R^3}$ (**20**) led to a significant loss in IR selectivity (IR/ALK IC_{50} ratio of 16) as well as broader kinome selectivity (S(90) 0.12),[17] showcasing the importance of a substituent on the 4-position of the **B** ring as a key selectivity determinant. The morpholine

Table 3
Biological data for compounds **18**–**24**



Compds	R^2	R^3	IC_{50}[a] (nM)			S(90) [b]
			ALK enzyme	ALK cell	IR enzyme	
18	MeO–		4	30	1380	0.03
19	H		49	–	504	–
20	MeO–	H	4	65	62	0.12
21	MeO–	$-C(O)NH_2$	6	65	573	0.11
22	MeO–	$-O(CH_2)_2OMe$	4	40	351	0.09
23	MeO–	–CN	7	65	233	0.06
24	MeO–	–OMe	5	40	189	0.11

[a] IC_{50} values are reported as the average of at least two separate determinations.
[b] Kinase selectivity was determined using the Ambit Bioscience KINOMEscan™ technology, and is expressed as S(90), the fraction of kinases inhibited >90% when screened at 1 μM across a panel of 256 kinases.

Table 4
Pharmacokinetic parameters of **18** in Sprague–Dawley rats and in CD-1 mice

	PK parameters	S–D rat[a]	CD-1 mouse
iv	Dose[b] (mg/kg)	1	1
	$t_{1/2}$ (h)	0.8 ± 0.05	0.7
	AUC_{0-t} (ng·h/mL)	1019 ± 91	830
	Vd (L/kg)	1.1 ± 0.1	1.2
	CL (mL/min/kg)	16 ± 1.5	20
po	Dose[b] (mg/kg)	5	10
	C_{max} (ng/mL)	306 ± 53	1192
	t_{max} (h)	3.3 ± 0.7	0.5
	$t_{1/2}$ (h)	n.d.	3.1
	AUC_{0-t} (ng·h/mL)	1395 ± 281	3895
	F (%)	30 ± 6	64

[a] Values are means of data from three animals.
[b] Formulated as solutions in: 3% DMSO, 30% Solutol, 67% PBS (iv); 100% PEG400 (po).

replacements in **21**–**24** afford lower molecular weight and somewhat increased polarity, while maintaining acceptable levels of ALK activity and IR selectivity. Noteworthy, the IR activity is related to the size of these functional groups.

Compared to **18**, **21**–**24** were 2–3 times less selective against the tested kinome panel (S(90) 0.06–0.11) and showed suboptimal rat PK data (oral bioavailability *F* in the 0–12% range, low exposures and short iv half-lives). Inhibitor **18** had acceptable pharmacokinetics in both rat and mouse (Table 4) for in vivo evaluation.

In cell proliferation assays, **18** demonstrated concentration dependent growth inhibition in ALK-positive ALCL cell lines Karpas-299 (calculated $IC_{50} \sim 160$ nM) and Sup-M2 (calculated $IC_{50} \sim 80$ nM), while it had a minimal effect on ALK-negative K562 cells up to 1000 nM tested.

Based upon the potency against ALK in enzyme and cellular assays (inhibition of phosphorylation and proliferation) combined with the promising oral PK, **18** was evaluated in a single-dose PK/PD experiment (30 mg/kg, po) in mice bearing ALK-positive tumor xenografts. **18** provided sustained inhibition of NPM–ALK autophosphorylation (75% inhibition for up to 12 h post-dose). Plasma concentrations of 3–5 μM were achieved out to 12 h with comparable levels observed in tumors. Mouse plasma protein binding of 97.2% rendered unbound concentrations of **18** of 90–130 nM, approximately 3–4-fold above the cellular IC_{50} of 30 nM. Consistent with the PK/PD response, dose-dependent antitumor efficacy in the Sup-M2 tumor xenograft model in SCID mice was

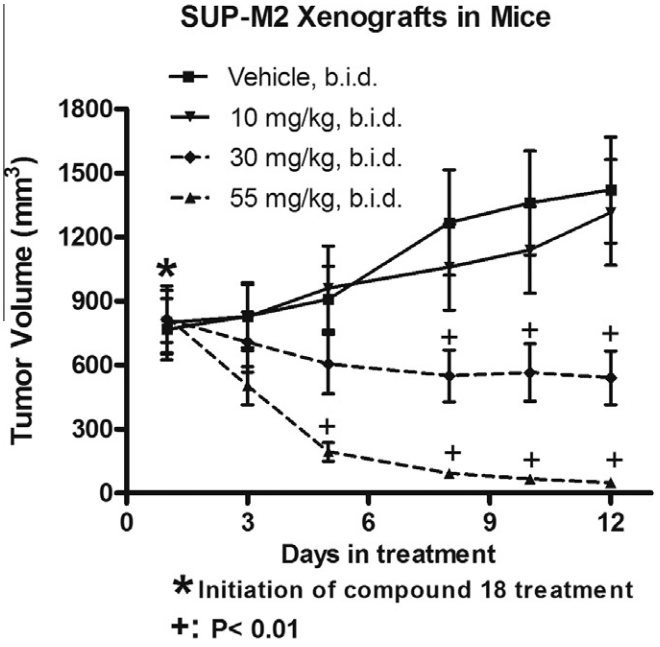


Figure 2. Antitumor efficacy of **18**, dosed po as a solution in PEG400.

obtained (Fig. 2). **18** exhibited tumor regressions at 30 mg/kg and 55 mg/kg b.i.d., po doses, at which it was well tolerated. No overt toxicity or body weight loss was observed.

Further profiling for potential off-target liabilities demonstrated that **18** was selective with respect to a panel of 64 receptors; inhibition levels at 10 μM were <50%, except for 5HT-1A (67% at 10 μM).[18] In a functional patch clamp assay to assess activity against the hERG ion channel, **18** displayed an $IC_{50} = 3.9$ μM.[19] Against the major CYP isoforms, minimal inhibition for CYP1A2 and 2D6 was seen (IC_{50} >20 μM), however, inhibition of the 2C9, 2C19, and 3A4 isoforms was 1.2, 0.8, 2.0 μM, respectively.

In summary, the synthesis and biological characterization of novel benzazepine-derived 2,4-diaminopyrimidines were described. The research efforts outlined above resulted in identification of potent and highly selective (including against IR) ALK inhibitors. Orally bioavailable analogues (exemplified by the tool compound **18**) from this series demonstrated in vivo efficacy in ALCL tumor models.

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