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2,4-Diaminopyrimidine inhibitors of c-Met kinase bearing benzoxazepine anilines

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ABSTRACT

Elaboration of the SAR around a series of 2,4-diaminopyrimidines led to a number of c-Met inhibitors in which kinase selectivity was modulated by substituents appended on the C4-aminobenzamide ring and the nature of the C2-aminoaryl ring. Further lead optimization of the C2-aminoaryl group led to benzoxazepine analogs whose pharmaceutical properties were modulated by the nature of the substituent on the benzoxazepine nitrogen. Tumor stasis (with partial regressions) were observed when an orally bioavailable analog was evaluated in a GTL-16 tumor xenograft mouse model. Subsequent PK/PD studies suggested that a metabolite contributed to the overall in vivo response.

Aberrant expression of hepatocyte growth factor/scatter factor (HGF/SF) and its endogenous receptor, c-Met, is associated with the development and poor prognosis of a wide range of solid tumors and implicated in the resistance to conventional therapies.[1,2] Given the role of c-Met in mediating the growth, invasion, angiogenesis and survival of multiple solid and hematological cancers, and functioning as a 'master switch in cancer progression',[3] the HGF/c-Met axis is an attractive pharmacological target for oncology drug discovery efforts.[4]

The Novartis FAK inhibitor (NVP-TAE 226, **1**) has been reported to possess sub-micromolar activity against c-Met.[5] Initial screening showed that C4-benzamide analogs provided c-Met activity.[6] Furthermore, removal of the methoxy substituent on the C2 aniline in **1** improved potency by 25-fold but at an expected cost in selectivity.[7] Our first set of compounds focused on rescuing selectivity by elaborating the C4-benzamide and utilizing a C2-benzo[*d*]azepine aniline to mimic the basic amine of the morpholino-aniline (Fig. 1). Analogs were evaluated in a c-Met enzymatic assay and in a c-Met overexpressing human gastric carcinoma cell line (GTL-16) assay and countered-screened against the insulin receptor (IR).[8,9] Select compounds were also evaluated for global kinase selectivity using Ambit Bioscience KINOMEscanTM technology,[10] expressed as an S(90) value which reflects the fraction of kinases inhibited >90% when screened at 1 μM across a panel of >250 kinases.

Synthesis of the 2-chloro-4-anilino pyrimidine was achieved as shown in Scheme 1. Isatoic anhydrides were reacted with methylamine followed by addition into 2,4,5-trichloropyrimidine to afford the dichloropyrimidines **4a–d**. The corresponding C2 aniline was prepared from benzo[*d*]azepine **5** by a straightforward three-step sequence. Acid-catalyzed coupling afforded the target compounds 7a–**d**.

This initial set of analogs (**7a–d**) demonstrated potent inhibition of c-Met activity (Table 1). Inhibition of the insulin receptor (IR) was significant for **7a** (X = H), but was strongly attenuated by incorporation of a substituent at the 3-position of the benzamide. Cellular activity suffered when chlorine (**7b**) and methyl (**7c**) groups were incorporated, but fluorinated analog (**7d**) showed excellent cellular activity (IC_{50} = 19 nM). The relative size of the benzamide C3 substituent correlated with selectivity, with the smallest, X = H, showing greater pan-kinase activity, and the larger, X = Cl or Me, showing high selectivity. Based on its moderate kinase selectivity and high potency in the cellular assay, the fluorobenzamide C4 aniline was selected for further optimization.

The C2-benzo[*d*]azepine lacked substitution in the benzylic positions, and it was postulated that a C2 aniline with branching at the 3- or 4-position could improve kinase selectivity. A substituted 1,5-benzoxazepine (such as **14** and **15**) would furnish an

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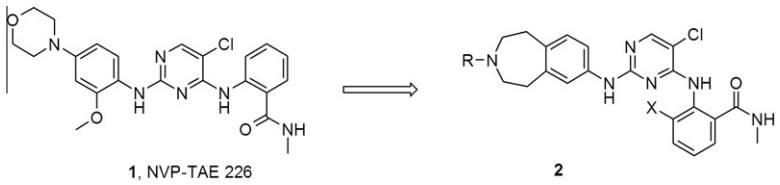
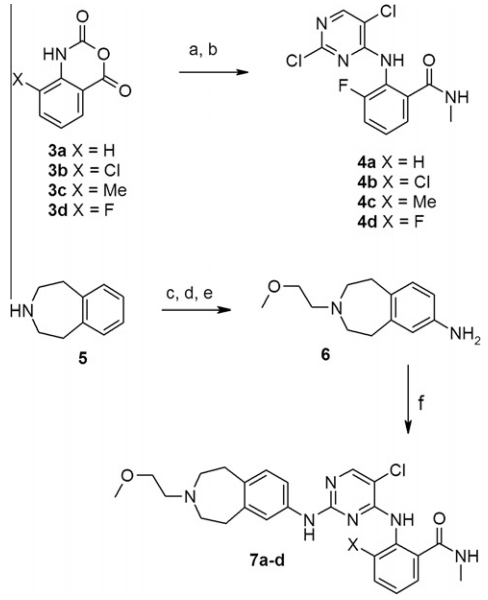


Figure 1. Novartis FAK inhibitor and initial targets **2**.



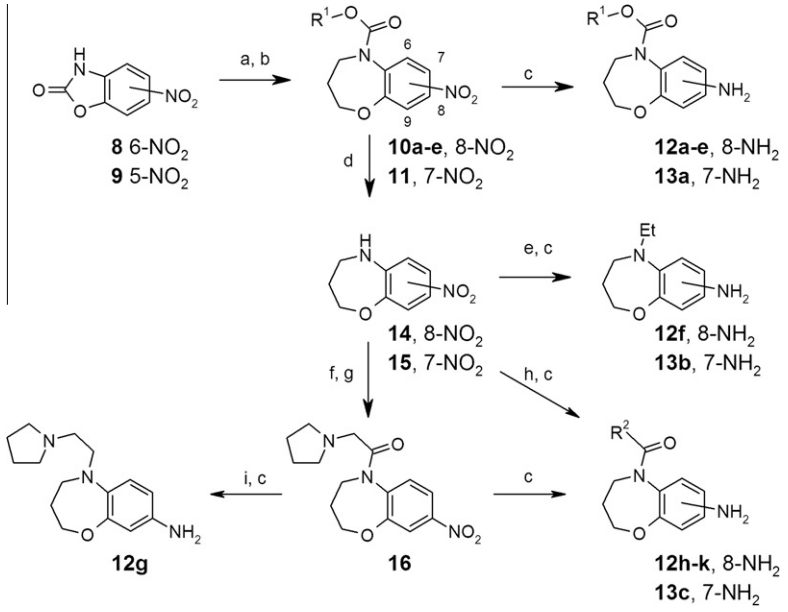
Scheme 1. Reagents: (a) $MeNH_2$, THF; (b) 2,4,5-trichloropyrimidine, DIEA, NMP, 100 °C; (c) nitric acid; (d) bromoethyl methyl ether, K_2CO_3; (e) H_2, Pd/C; (f) **4a–d**, camphorsulfonic acid, 2-BuOH, reflux.

aniline nitrogen handle to facilitate SAR elaboration in order to test this hypothesis. Following a known procedure,[11] alkylation of 6- or 5-nitrobenzoxazolidone (**8** or **9**) followed by a ring-expanding treatment with alkoxide generated benzoxazepine carbamates (**10a–e**, **11**, respectively, Scheme 2). Simple hydrogenation furnished the carbamate capped anilines. Hydrolysis of the ethyl carbamates, (**10b**, **11**) yielded intermediates that ultimately provided *N*-alkyl (**12f–g**, **13b**) or *N*-acyl (**12h–k**, **13c**) benzoxazepine anilines in 2–4 steps.

Table 1
Benzazepine analog SAR

Analog	X	c-Met enzyme IC_{50} (nM)	c-Met cell IC_{50} (nM)	IR enzyme IC_{50} (nM)	Ambit S(90)
7a	H	41	220	20	0.58
7b	Cl	24	550	>10,000	0.06
7c	Me	26	1400	>3000	0.06
7d	F	8.0	19	230	0.35

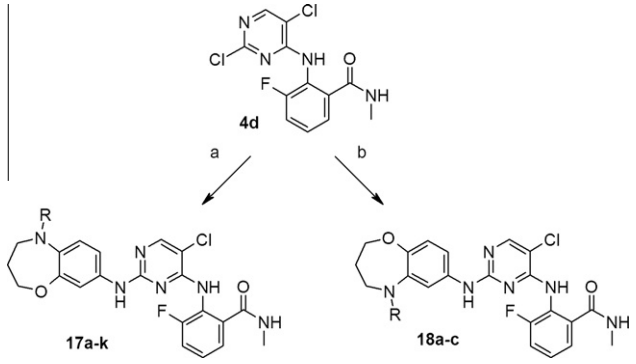
Acid-catalyzed coupling of the dichloropyrimidine **4d** with the 5-substituted 8- or 7-aminobenzoxazepine furnished targets **17a–k** or **18a–c**, respectively (Scheme 3).[12] Table 2 summarizes the c-Met inhibitory data (enzyme and cell), as well as the selectivity data [IR and the kinome (Ambit S(90) values at 1 μM)] for these benzoxazepines. The aliphatic-capped analogs (**17f**, **17g**) were the most potent in the cellular assay, but gave unacceptably low oral exposure in rat PK (5 mg/kg PO $AUC_{0-\infty}$ <100 ng h/mL). All of the carbamate-capped analogs (**17a–e**) had similar cellular activity (28–53 nM) and kinase selectivity [S(90) = 0.14–0.17]. Carbamate **17e**, featuring an aliphatic amine, had the least amount of activity against IR, but also displayed low oral exposure in rat PK. Analog **17b**, however, furnished acceptable rat PK (1 mg/kg IV, 5 mg/kg PO; IV $t_{1/2}$ = 1.2 h; CL = 15.3 mL/min/kg; IV $AUC_{0-\infty}$ = 1224 ng h/mL; PO



Scheme 2. Reagents: (a) 1-bromo-3-chloropropane, K_2CO_3, acetone; (b) $NaOR^1$, NMP; (c) H_2, Pd/C; (d) NaOH; (e) CH_3CHO, $NaBH(OAc)_3$; (f) $ClCH_2COCl$, Et_3N, DMAP; (g) pyrrolidine; (h) TFAA or R^2COCl, pyr, DMAP; (i) BH_3·THF; See Table 2 for R group descriptions in analogs **12a–e**, **12h–k**, **13a**, **13c**.

Table 2
Benzoxazepine analog SAR

Analog	R	c-Met enzyme IC_{50} (nM)	c-Met cell IC_{50} (nM)	IR enzyme IC_{50} (nM)	Ambit S(90)
17a	CO_2Me	32	46	550	0.16
17b	CO_2Et	25	36	1000	0.17
17c	CO_2-*i*-Pr	47	53	820	0.14
17d	$CO_2(CH_2)_2OMe$	81	50	800	0.14
17e	$CO_2(CH_2)_3NMe_2$	45	28	1400	0.16
17f	Et	14	18	370	0.22
17g	$(CH_2)_2$-pyrrolidine	21	22	300	0.25
17h	H	42	57	560	0.21
17i	Ac	28	30	500	0.17
17j	$(CO)CH_2$-pyrrolidine	44	19	2300	0.12
17k	$(CO)CH_2OMe$	13	51	930	0.15
18a	CO_2Et	140	440	NT	NT
18b	Et	120	41	490	0.21
18c	Ac	17	49	1200	0.20



Scheme 3. Reagents: (a) **12a**–**k**, camphorsulfonic acid, 2-BuOH, reflux; (b) **13a**–**c**, camphorsulfonic acid, 2-BuOH, reflux.

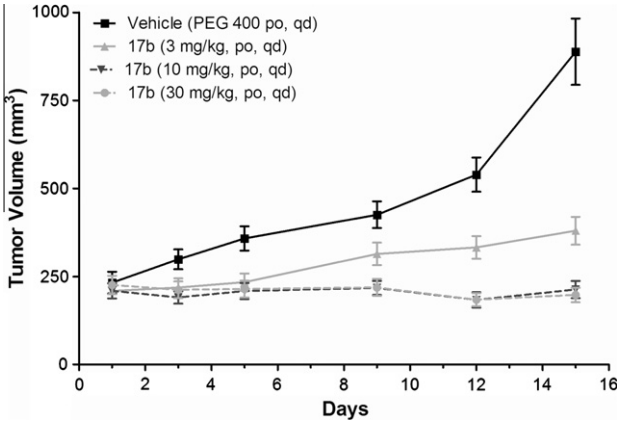


Figure 2. Effect of **17b** on GTL-16 tumor xenograft.

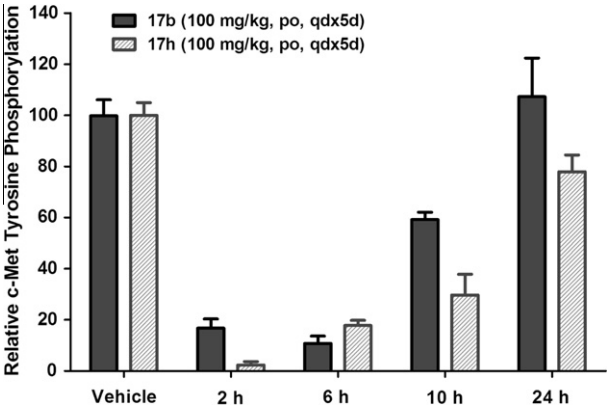


Figure 3. Inhibition of c-Met phosphorylation by **17b** and **17h** on day 5 of dosing, 1 h post dose.

$AUC_{0-\infty}$ = 913 ng h/mL; %*F* = 15%) and was selected for in vivo efficacy experiments. Amide capped analogs (**17i–k**) fared poorly in rat PK studies. In the 7-aminobenzoxazepine series, carbamate **18a** was significantly less active in the cellular assay and **18b** and **18c** displayed poor oral exposure in rat PK.

Oral administration of **17b** at 3, 10 or 30 mg/kg (po, qd) in a 15-day, GTL-16 xenograft tumor model (Fig. 2) showed tumor stasis at the higher doses, with partial regressions in 40% and 50% of the test animals, respectively. At 3 mg/kg, 60% tumor growth inhibition was achieved. All dosing regimens were well tolerated, with no significant reduction in body weight. Paradoxically, tumor and plasma samples taken at day 5 showed low levels of **17b**. For example, at a 3 mg/kg dose, tumor levels of **17b** barely exceeded its cellular c-Met IC_{50} (35 nM or 18 ng/mL). A subsequent in vitro metabolite ID study (i.e., incubation of **17b** with mouse liver microsomes) revealed that **17h** was a primary metabolite (carbamate hydrolysis). Since **17h** had comparable c-Met cellular activity as **17b**, it was possible that the observed in vivo efficacy could have been driven, to varying degrees, by these two agents.

A five-day PK/PD experiment was conducted to simultaneously assess levels of **17b** and **17h** in plasma and tumor samples after dosing GTL-16 tumor xenograft-bearing nude mice with **17b** or **17h** at 100 mg/kg (po, qd, Figs. 3 and 4). The PD response for each analog was similar, with **17h** exhibiting slightly greater knockdown of c-Met phosphorylation over the 24 h period following the last dose (Fig. 3). Whether dosed as **17h** or **17b**, similar tumor levels of **17h** were observed and these remained above 200 ng/g over 10 h (Fig. 4b). Although plasma levels of **17b** quickly fell below its cellular IC_{50} value after 2 h (Fig. 4), tumor levels remained constant from 2 to 10 h (125 ng/g). Thus the original tumor growth inhibition (Fig. 2) might not solely be attributed to **17b**, and presumably metabolites such as **17h** contributed to the overall biological response. The lack of a durable PD effect at a relatively high dose (100 mg/kg) coupled with generally poor rat PK properties of **17h**, such as high clearance (CL = 34 mL/min/kg) and a short half-life (IV $t_{1/2}$ = 0.3 h), reduced interest in its further development.

In conclusion, potent inhibitors of c-Met were prepared containing a benzoxazepine aniline at C2 of the 2,4-diaminopyrimidine scaffold; selectivity was modulated by incorporation of fluorine on the C4 aniline. Analog **17b** was orally bioavailable

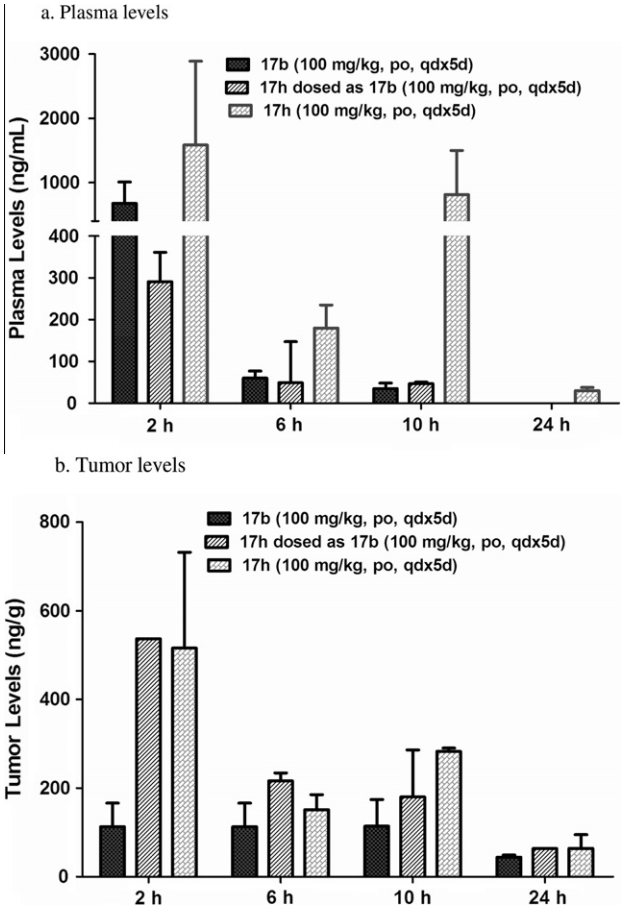


Figure 4. PK data for **17b** and **17h** on day 5 of dosing, 1 h post dose.

(rat) and demonstrated in vivo tumor stasis in a GTL-16 tumor xenograft mouse model. Oral administration of **17b** in mice led to formation of an equipotent metabolite (**17h**), which contributed to overall tumor growth inhibition.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.12.013.

References and notes

1. Birchmeier, C.; Birchmeier, W.; Gherardi, E.; Vande Woude, G. F. *Nat. Rev. Mol. Cell Biol.* **2003**, *4*, 915.
2. Christensen, J. G.; Burrows, J.; Salgia, R. *Cancer Lett.* **2005**, *225*, 1.
3. Mazzone, M.; Comoglio, P. M. *FASEB J.* **2006**, *20*, 1611.
4. For reviews of c-Met inhibitors in development, see: (a) Liu, X.; Newton, R. C.; Scherle, P. A. *Trends Mol. Med.* **2010**, *16*, 37; (b) Underiner, T. L.; Herbertz, T.; Miknyoczki, S. J. *Anti-Cancer Agents Med. Chem.* **2010**, *10*, 7.
5. Liu, T.-J.; LaFortune, T.; Honda, T.; Ohmori, O.; Hatakeyama, S.; Meyer, T.; Jackson, D.; de Groot, J.; Yung, W. K. A. *Mol. Cancer Ther.* **2007**, *6*, 1357.
6. Weinberg, L. R.; Albom, M. S.; Angeles, T. S.; Diebold, J. L.; Husten, J.; McHugh, R. J.; Lisko, J. G.; Milkiewicz, K. L.; Murthy, S.; Ott, G. R.; Ruggeri, B. A.; Theroff, J. P.; Tripathy, R.; Underiner, T. L.; Zificsak, C. A.; Dorsey, B. D. *Bioorg. Med. Chem. Lett.* **2010**. doi:10.1016/j.bmcl.2010.11.045.
7. Galkin, A. V.; Melnick, J. S.; Kim, S.; Hood, T. L.; Li, N.; Li, L.; Xia, G.; Steensma, R.; Chopiuk, G.; Jiang, J.; Wan, Y.; Ding, P.; Liu, Y.; Sun, F.; Schultz, P. G.; Gray, N. S.; Warmuth, M. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 270.
8. Assay descriptions can be found in the Supplementary data.
9. As a key regulator of glucose metabolism, selectivity against IR was desired to be >10-fold in the enzymatic assays.
10. Fabian, M. A.; Biggs, W. H., III; Treiber, D. K.; Atteridge, C. R.; Azimioara, M. D.; Benedetti, M. G.; Carter, T. A.; Ciceri, P.; Edeen, P. T.; Floyd, M.; Ford, J. M.; Galvin, M.; Gerlach, J. L.; Grotzfeld, R. M.; Herrgard, S.; Insko, D. E.; Insko, M. A.; Lai, A. G.; Lelias, J.-M.; Mehta, S. A.; Milanov, Z. V.; Velasco, A. M.; Wodicka, L. M.; Patel, H. K.; Zarrinkar, P. P.; Lockhart, D. J. *Nat. Biotechnol.* **2005**, *23*, 329.
11. Sam, J.; Valentine, J. L.; Aboul-Enein, M. N. *J. Pharm. Sci.* **1971**, *60*, 1370.
12. Compound **12h**, $R^2 = CF_3$, was coupled with **4d** to furnish the trifluoroacetyl capped target, which was treated in situ with K_2CO_3/MeOH to furnish **17h**.