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Fused bicyclic derivatives of 2,4-diaminopyrimidine as c-Met inhibitors

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ABSTRACT

The HGF-c-Met signaling axis is an important paracrine mediator of epithelial–mesenchymal cell interactions involving the regulation of multiple cellular activities including cell motility, mitogenesis, morphogenesis, and angiogenesis. Dysregulation of c-Met signaling (e.g., overexpression or increased activation) is associated with the development of a wide range of tumor types; thus, inhibiting the HGF-c-Met pathway is predicted to lead to anti-tumor effects in many cancers. Elaboration of a 2-arylaminopyrimidine scaffold led to a series of potent c-Met inhibitors bearing a C4-2-amino-*N*-methylbenzamide group. Specifically, a series of C2-benzazepinone analogs demonstrated potent inhibition of c-Met in enzymatic and cellular assays. Kinase selectivity could be tuned by varying the nature of the alkyl group on the benzazepinone nitrogen.

Mesenchymal epithelial transition factor (c-Met) kinase and its natural ligand, hepatocyte growth factor (HGF) are involved in signaling pathways for embryological development, wound healing, tissue regeneration, angiogenesis, proliferation, survival, scattering, and morphogenic differentiation. The pathway regulates a series of intracellular downstream events in many different cell types that lead to cell growth, motility, and invasion.

Due to the c-Met/HGF role of regulation of cellular motility, cells expressing high levels of HGF and/or c-Met are likely to be metastatic.[1,2] The cells can become solid tumors in the liver, breast, pancreas, lung, kidney, bladder, ovary, brain, prostate, and gallbladder.[1,2]

A number of Type I (DFG-in) and Type II (DFG-out) c-Met inhibitors are currently being evaluated clinically and several Type I inhibitors which are currently in clinical trials are shown in Figure 1.[3]

In connection with other work focused on optimizing a 2,4-diaminopyrimidine scaffold to inhibit ALK, it was discovered that compounds with an aminobenzamide appended at C4 consistently demonstrated potent c-Met inhibition (Fig. 2).[4,5] This putative Type I kinase inhibitor platform served as a starting point in a medicinal chemistry effort to optimize c-Met cellular activity while improving overall kinase selectivity. This Letter describes the synthesis and c-Met SAR around a series of diaminopyrimidine analogs bearing a C4-aminobenzamide and a C2-benzazepinone substituent and how kinase selectivity can be tuned by varying the nature of these appendages (Fig. 2).

In general, these diaminopyrimidine targets were prepared by first coupling 2-aminobenzamide analogs **2**, derived from the corresponding isatoic anhydrides **1**, with trichloropyrimidine to afford aminopyrimidines **3**; these intermediates were then coupled to aminobenzazepin-2-ones **4** or **5** (Scheme 1).

Aminobenzazepin-2-one isomers (**4** and **5**) were prepared as described in Schemes 2 and 3. Beckmann rearrangement of the oxime derived from tetralone **6** produced benzazepinone **8**.[5] Nitration of **8a** or **8b** followed by either reduction or alkylation and reduction afforded intermediates **4a–d**.

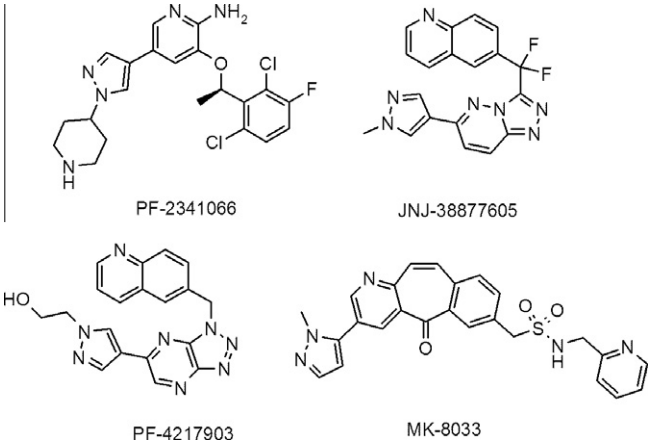

Figure 1. Type I c-Met inhibitors in clinical trials.

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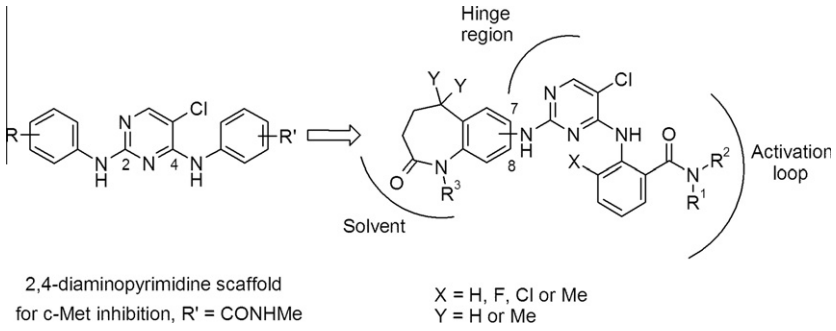
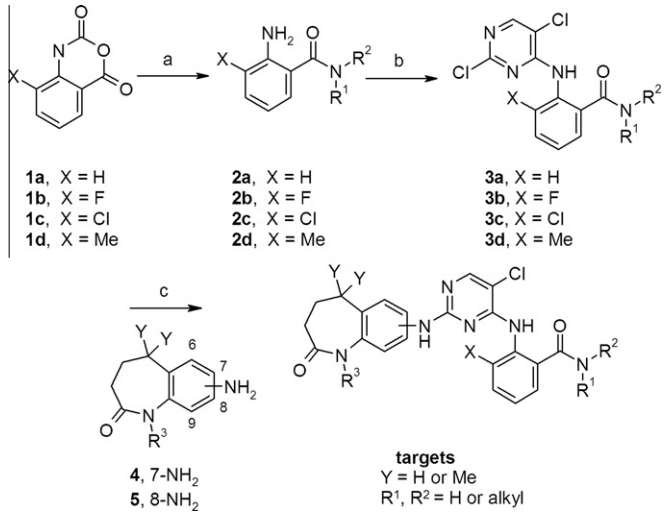
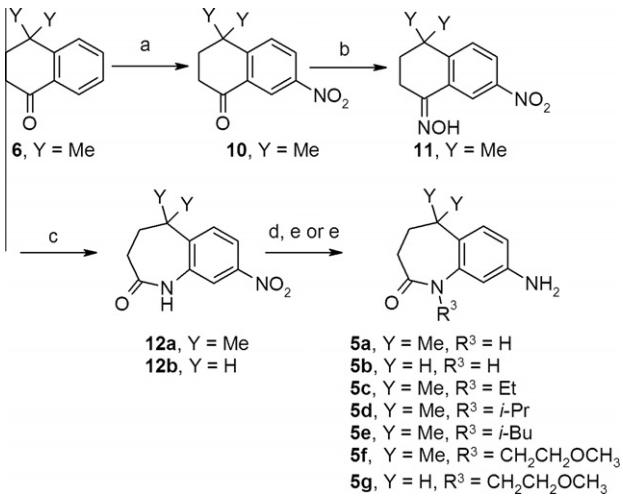


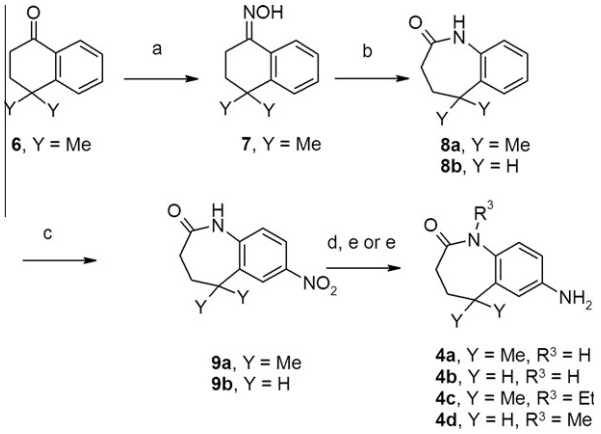
Figure 2. Diaminopyrimidine kinase inhibitor scaffold.



Scheme 1. Target synthesis. Reagents and conditions: (a) R^1R^2NH, THF; (b) 2,4,5-trichloropyrimidine, DIPEA, NMP, 100 °C; (c) DL-10-camphorsulfonic acid, IPA, μW at 120 °C.



Scheme 3. Synthesis of 8-amino-1,3,4,5-tetrahydro-benzo[*b*]azepin-2-one (**5**). Reagents and conditions: (a) H_2SO_4, HNO_3; (b) NaOAc, $NH_2OH{\cdot}HCl$, EtOH, water, reflux; (c) PPA, 125 °C; (d) R^3X, Cs_2CO_3, DMF; (e) 10% Pd/C, $N_2H_4{\cdot}H_2O$, EtOH, 60 °C.



Scheme 2. Synthesis of 7-amino-1,3,4,5-tetrahydro-benzo[*b*]azepin-2-one (**4**). Reagents and conditions: (a) NaOAc, $NH_2OH{\cdot}HCl$, EtOH, water, reflux; (b) PPA, 125 °C; (c) H_2SO_4, HNO_3; (d) R^3X, Cs_2CO_3, DMF; (e) 10% Pd/C, $N_2H_4{\cdot}H_2O$, EtOH, 60 °C.

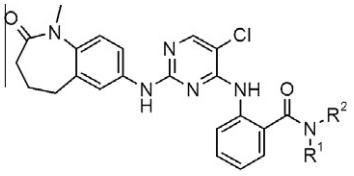
A similar synthesis was carried out to make the isomeric 8-amino-1,3,4,5-tetrahydro-benzo[*b*]azepin-2-ones **5**, and is shown in Scheme 3. Nitration of tetralone **6** afforded **10**. A Beckmann rearrangement of the corresponding oxime **11** produced the benzazepinone **12a**. Both **12a** and **12b**[6] were either reduced or alkylated and then reduced to give anilines **5**.[5]

In addition to optimizing for c-Met inhibition, medicinal chemistry efforts were directed at enhancing selectivity against the insulin receptor kinase (IR)[7] in particular, and against a broad panel of kinases in general. Kinase selectivity was determined using Ambit Bioscience KINOMEscan™ technology and is expressed as an *S*(90) value, which indicates the fraction of kinases inhibited >90% when screened at 1 μM across a panel of 256 kinases.[8]

The benzamide SAR is summarized in Table 1. Increasing size of the secondary amide group (**13** vs **16**) led to reduced c-Met inhibitory activity. In this series, analog **14** was the most potent cellular c-Met inhibitor and demonstrated fourfold selectivity for c-Met over IR. Nonetheless, this analog demonstrated >90% inhibition of 151 out of 256 kinases when screened at 1 μM. Interestingly, the propargyl benzamide analog **17** was also quite active while the corresponding *N*-Me tertiary amide **18** was >20-fold less active. Optimization thus focused on analogs of methylbenzamide **14**.

Kinase selectivity was dramatically improved upon incorporation of a C3 substituent on the benzamide ring (e.g., a halogen or methyl group, Table 2). For example, the *S*(90) value fell from 0.57 for analog **19** (X = H) to 0.02 for analog **21** (X = Cl). Although analog **21** had improved selectivity, it had an unacceptably high GTL-16 cell IC_{50}. Analog **20** (X = F) represented a better compromise; although the IR/c-Met IC_{50} ratio remained at 4, the *S*(90) value fell to 0.31 with no significant impact on c-Met potency or cell translation. The C4-substituent was fixed as 2-amino-3-fluoro-*N*-methylbenzamide, and medicinal chemistry focused on optimizing the C2-benzazepin-2-one.

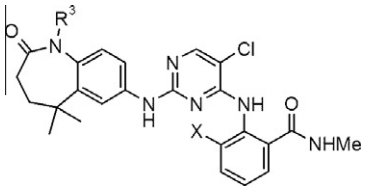
Table 1
c-Met SAR and kinase selectivity of benzamide analogs **13**–**18**



Compd	R^1	R^1	IC_{50}[a] (nM)				
			c-Met enzyme	c-Met cell[b]	IR enzyme	IR/c-Met enzyme	$S(90)$[c]
13	H	H	7	85	4	0.6	0.69
14	Me	H	6	10	23	4	0.59
15	*n*-Pr	H	73	202	11	0.2	NT[d]
16	*n*-Bu	H	161	NT[d]	34	0.2	NT[d]
17	CH_2CCH	H	17	38	8	0.5	0.42
18	CH_2CCH	Me	484	NT[d]	NT[d]	NT[d]	0.34

[a] Average of at least two separate determinations; see Supplementary data for assay conditions.
[b] GTL-16 cells.
[c] Fraction of 256 kinases inhibited >90% when screened at 1 μM.[8]
[d] Not tested.

Table 2
c-Met SAR and kinase selectivity of benzamide analogs **19**–**23**



Compd	X	R^3	IC_{50}[a] (nM)				
			c-Met enzyme	c-Met cell[b]	IR enzyme	IR/c-Met enzyme	$S(90)$[c]
19	H	H	54	80	30	0.6	0.57
20	F	H	45	102	200	4	0.31
21	Cl	H	159	1652	>6500	>40	0.02
22	Me	H	203	NT[d]	NT[d]	NT[d]	0.04
23	F	Et	56	64	334	6	0.23

[a] Average of at least two separate determinations; see Supplementary data for assay conditions.
[b] GTL-16 cells.
[c] Kinase selectivity was determined using the Ambit Bioscience KINOMEscan™ technology.
[d] Not tested.

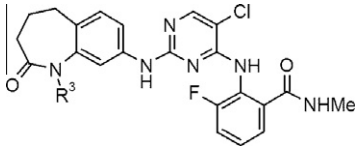
Incorporation of an ethyl group on the 7-aminobenzazepinone nitrogen **23** maintained c-Met cell potency (cell IC_{50} = 64 nM) and improved kinase selectivity ($S(90)$ = 0.23, Table 2). However, the IR IC_{50} was modestly affected and the IR/c-Met IC_{50} ratio remained <10.

Initial results indicated that the IR/c-Met ratio was improved in the 8-aminobenzazepinone series (Table 3). For example, benzazepinone **25**, R^3 = methoxyethyl, maintained cellular c-Met inhibition and overall kinase selectivity but had increased separation from IR activity (IR/c-Met IC_{50} ratio = 115). This SAR was more fully explored in the 8-amino-5,5-dimethylbenzazepin-2-one series (Table 4); c-Met inhibition fell while kinase selectivity (i.e., $S(90)$ values) improved with increasing α-branching of the lactam substituent (R^3). In this series, analog **27** (R^3 = Et) possessed the best balance of c-Met cellular potency (cell IC_{50} = 44 nM), selectivity against IR (IR/c-Met IC_{50} ratio = 73), and kinase selectivity [$S(90)$ = 0.17] see Supplementary data for a list of kinases inhibited by this compound greater than 90% at1 μM).

Analog **24** demonstrated modest IV and oral exposure in rat (1 mg/kg IV, 5 mg/kg PO: IV $t_{1/2}$ = 0.7 h; CL = 15 mL/min/kg; IV $AUC_{0-\infty}$ = 1261 ng h/mL; PO $AUC_{0-\infty}$ = 592 ng h/mL; %F = 8%). Analog **26**, with its putatively metabolic benzylic sites protected with methyl groups demonstrated lower oral exposure (1 mg/kg IV, 5 mg/kg PO: IV $t_{1/2}$ = 0.3 h; CL = 16 mL/min/kg; IV $AUC_{0-\infty}$ = 1057 ng h/mL; PO $AUC_{0-\infty}$ = 46 ng h/mL; %F <1%), and analog **27** had even lower IV and oral coverage. Current efforts are directed at improving the PK properties of these analogs.

In summary, a series of 2,4-diaminopyrimidine analogs bearing a C4-*N*-methylbenzamide and a C2-benzazepin-2-one was prepared and representative compounds were shown to be potent inhibitors of c-Met kinase. Kinase selectivity could be increased without compromising c-Met cellular activity by incorporating a fluorine atom at the C3 position of the pendant aminobenzamide. Further improvement in kinase selectivity in general, and over IR kinase in particular, could be realized by substitution of the benzazepinone nitrogen. Thus, the $S(90)$ values dropped from 0.57

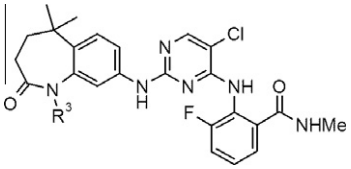
Table 3
c-Met SAR and kinase selectivity of 8-aminobenzazepinone analogs **24**–**25**



Compd	R^3	IC_{50}[a] (nM)				
		c-Met enzyme	c-Met cell[b]	IR enzyme	IR/c-Met enzyme	$S(90)$[c]
24	H	30	99	369	12	0.24
25	$(CH_2)_2OMe$	10	61	1150	115	0.25

[a] Average of at least two separate determinations; see Supplementary data for assay conditions.
[b] GTL-16 cells.
[c] Kinase selectivity was determined using the Ambit Bioscience KINOMEscan™ technology.

Table 4
c-Met SAR and kinase selectivity of 8-amino-5,5-dimethylbenzazepinone analogs **26**–**30**



Compd	R^3	IC_{50}[a] (nM)				
		c-Met enzyme	c-Met cell[b]	IR enzyme	IR/c-Met enzyme	$S(90)$[c]
26	H	54	10	153	3	0.29
27	Et	10	44	730	73	0.17
28	*i*-Pr	376	NT[d]	404	1	NT[d]
29	*i*-Bu	84	214	840	10	0.07
30	$(CH_2)_2OMe$	18	85	231	13	0.23

[a] Average of at least two separate determinations; see Supplementary data for assay conditions.
[b] GTL-16 cells.
[c] Kinase selectivity was determined using the Ambit Bioscience KINOMEscan™ technology.
[d] Not tested.

for our initial lead compound **19** to 0.17 for substituted analog **27** and the IR/c-Met IC_{50} ratio increased from 0.6 to 73 without impacting c-Met cellular IC_{50} values (80 nM vs 44 nM, respectively).

Supplementary data

Supplementary data (biological assay conditions, the kinase profile for compound **27**, experimental procedures and spectral characterization) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.11.045.

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