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Discovery of substituted 6-pheny-3*H*-pyridazin-3-one derivatives as novel c-Met kinase inhibitors

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ABSTRACT

We report a series of phenyl substituted pyridazin-3-ones substituted with morpholino-pyrimidines. The SAR of the phenyl was explored and their c-Met kinase and cell-based inhibitory activity toward c-Met driven cell lines were evaluated. Described herein is a potent c-Met inhibitor by structural modification of the parent morpholino-pyridazinone scaffold, with particular focus on the phenyl and pyrimidine substituents.

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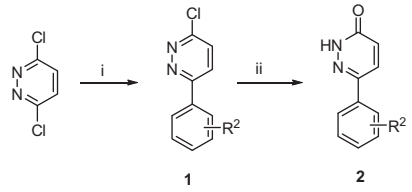
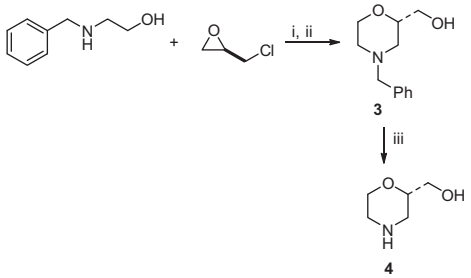
c-Met (mesenchymal epithelial transition factor) is the prototypic member of a subfamily of receptor tyrosine kinases and its ligand HGF (hepatocyte growth factor) have been received considerable attention and studied extensively as attractive therapeutic cancer targets.[1] c-Met and HGF are highly overexpressed in various cancer tissues, such as lung, gastric, renal, ovarian, prostate, and liver cancers.[2] Upon binding of HGF, c-Met dimerizes, transphosphorylates, and activates the signaling cascades for recruiting adaptor proteins, such as growth factor-bound protein 2 (Grb2), Grb2-associated-binding protein 1 (Gab1), PLC and SRC.[3] Constitutive activation of c-Met has been severely implicated in tumorigenic invasive growth, migration, proliferation, survival, angiogenesis, and metastasis by downstream signal transducers, such as MAPK, PIP3K, and STAT pathways.[4] In addition, the aberrant HGF/MET signaling cascade by activating mutations, gene rearrangements, bypass mechanisms, and interactions with other receptor families confers major key factors for acquiring resistance to EGFR and BRAF kinase inhibitors.[5] Overexpression of Met is strongly correlated with a metastatic activation of tumors and poor prognosis in a number of human cancers.[6]

Indeed, the regulation of c-Met activities in physiological conditions provides strong rational for targeting c-Met in cancer



Scheme 1. Reagents and conditions: (i) $PhB(OH)_2$, $Pd(dppf)_2Cl_2$, K_2CO_3, 90 °C, 3 h, 81%; (ii) AcOH, H_2O, 120 °C, 4 h, 83%.



Scheme 2. Reagents and conditions: (i) H_2O/^{i}PrOH, rt, 13 h; (ii) NH_4OH, rt, 3 h, 50% (2 steps); (iii) Pd/C, H_2, MeOH, 98%.

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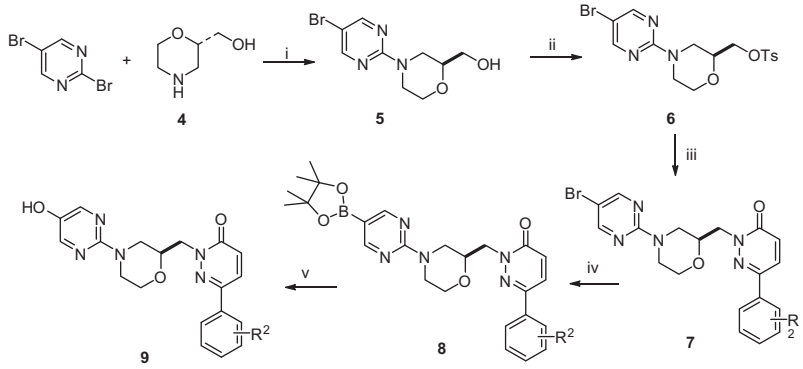
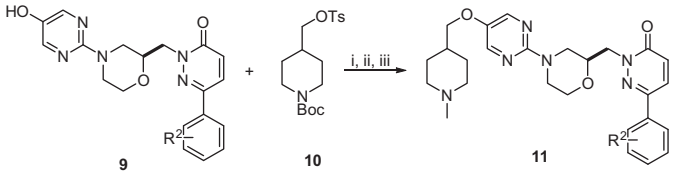


Scheme 3. Reagents and conditions: (i) $^{i}Pr_2NH$, EtOH, rt, 13 h, 87%; (ii) TsCl, DMAP, Et_3N, CH_2Cl_2, rt, 2 h, 99%; (iii) **2**, Cs_2CO_3, 50 °C, 13 h, 60%; (iv) 4,4,4′,4′,5,5,5′,5′-octamethyl-2,2′-bis(1,3,2-dioxaborolane), KOAc, dppf, dioxane, 80 °C, 13 h, 70%; (v) $NaBO_3$, H_2O, THF, 30%, (2 steps).



Scheme 4. Reagents and conditions: (i) K_2CO_3, DMF, 95 °C, 13 h, 50%; (ii) TFA, rt, 13 h, 52%; (iii) HCHO (35%), HCO_2H, 95 °C, 13 h, 61%.

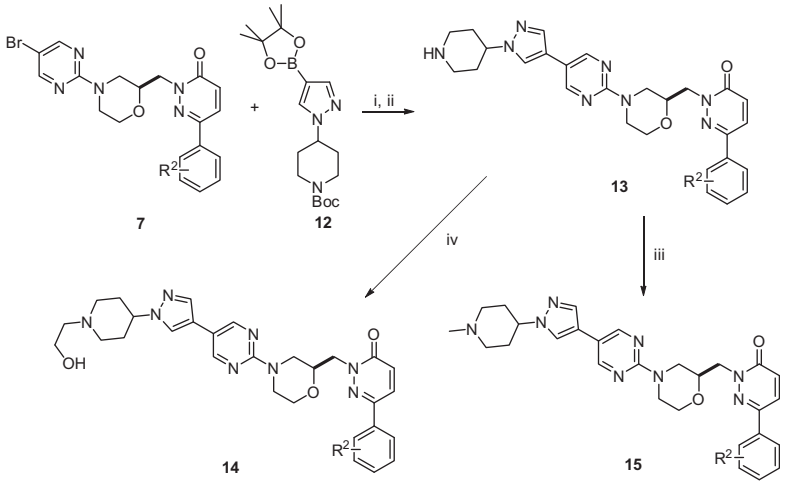
treatments. A number of c-Met kinase inhibitors have been reported and some of them reached the clinical trial.[7]

Recently, we have reported c-Met inhibitors and presented their biological activities.[8] In our efforts to discover novel c-Met kinase inhibitors, a class of compounds based (*S*)-2-((4-(5-bromopyrimidin-2-yl)morpholin-2-yl)methyl)-6-phenylpyridazin-3(2*H*)-one were synthesized and their biological activity was evaluated. Described herein is a series of novel pyridazinone-based inhibitors of c-Met kinases, which were substituted with morpholinopyrimidines.

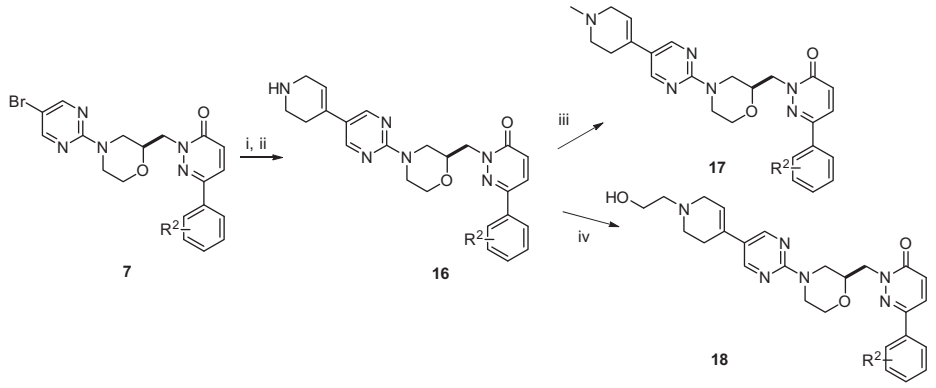
The synthesis of phenyl substituted pyridazinones **2** is outlined in Scheme 1 by convenient synthesis from commercially available 2,5-dichloropyridazine followed by palladium catalyzed cross-coupling reaction of the corresponding aromatic compounds. 2-Hydroxyethylmorpholine **4** can be easily furnished by starting with 2-(benzylamino)ethan-1-ol and epichlorohydrin to afford (*S*)-(4-benzylmorpholin-2-yl)methanol **3**. (*S*)-morpholin-2-ylmethanol **4** was obtained by simple deprotection of benzyl functionality of **3** (Scheme 2). Treatment of **4** with 2,5-dibromopyrimidine in the presence of diisopropylamine led to the intermediate **5**.

Versatile intermediate **7** was easily furnished by treatment of **6** with suitable precursor **2**, which was derived from **5** by simple tosylation.

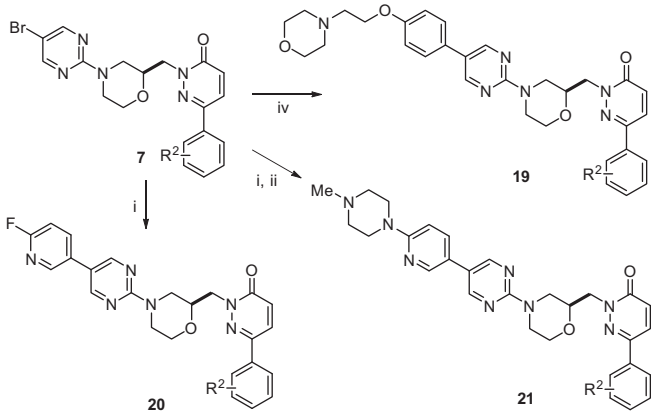
Successive treatment of bromide **7** to afford pinacolborane derivative **8** was accomplished by palladium-catalyzed cross coupling of bis(pinacolato) diboron compound as shown in Scheme 3. Hydroxyl group was introduced by $NaBO_3$ oxidation of **8** to afford **9**. The preparation of final *N*-methylpiperidine attached pyridazinone derivative **11** was outlined in Scheme 4. 5-Pyrimidinol was reacted with appropriate piperidine **10** followed by deprotection of Boc group, and subsequently the resulting piperidine was reacted with formaldehyde in the presence of formic acid to afford compound **11**. A variety of aromatic groups were introduced by cross-coupling of suitable aromatic borolane **12** to afford compound **13**, and subsequent treatment of 2-bromoethanol and formaldehyde afforded **14** and **15**, respectively (Scheme 5). A series of tetrahydropyridine derivatives was prepared from the appropriate alkylation of tetrahydropyridine **16** either in methylation condition or alkylation condition to afford **17** and **18**, respectively (Scheme 6). A series of pyridine and phenyl coupled compounds **19**, **20** and **21** varying in the side chain portion of pyridine and phenyl was



Scheme 5. Reagents and conditions: (i) $Pd(PPh_3)_2Cl_2$, CH_2Cl_2, DME/H_2O (3:1), 80 °C, 3 h, 64%; (ii) 4 M HCl in 1,4-dioxane, CH_2Cl_2, rt, 1 h, 90%; (iii) HCHO (35%), HCO_2H, 95 °C, 13 h, 60%; (iv) bromoethanol, K_2CO_3, DMF, 60 °C, 4 h, 55%.



Scheme 6. Reagents and conditions: (i) *N*-Boc-1,2,3,6-tetrahydropyridine-4-boronic acid pinacol ester, $Pd(dppf)_2Cl_2$, K_2CO_3, dioxane/H_2O (4: 1), 85 °C, 3 h, 90%; (ii) 4 M HCl/dioxane, CH_2Cl_2, rt, 4 h, 94%; (iii) HCO_2H, HCHO (35%), 95 °C, 13 h, 71%; (iv) 2-bromoethanol, K_2CO_3, 60 °C, 3 h, 77%.



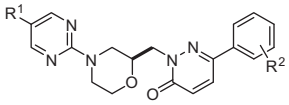
Scheme 7. Reagents and conditions (i) $Pd(dppf)_2Cl_2$, Cs_2CO_3, 6-fluoropyridin-3-yl boronic acid, 80 °C, 4 h, 70%; (ii) 1-methylpiperazinen, DIPEA, NMP, 150 °C, 13 h, 43%; (iii) morpholinoethanol, NaH, THF, rt, 3 h, 50%; (iv) 4-(2-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenoxy)ethyl) morpholine, Cs_2CO_3, $PdCl_2$, dimethoxyethane/H_2O (3:1), 80 °C, 2 h, 76%.

prepared from bromopyridine **7** with the appropriate boronic acid or boronic ester also by use of methods (vide supra, Scheme 7).

The in vitro c-Met kinase inhibitory activities toward substituents effect on R^1 and R^2 position of phenyl compartment were tested and the results are summarized in Table 1. To understand substituent effect of pyrimidine compartment, introduction of pyrazole and its derivatives (**12a**, **12b**, **12c**) were investigated on R^2 of phenyl substituents.

In cell cytotoxicity assay of *N*-methyl pyrazole moiety, one-digit micromolar range of inhibitory activity was observed in Hs746T, H1993, H3122 and U87MG cell lines (negative cell line) (**7a**). Subsequently, substitution of piperidine in place of methyl significantly improved in vitro enzyme inhibitory activities and cell proliferation inhibitory activities in Hs746T and H1993 cell lines (**13d**, **13e**). Of the phenyl substituents, 3-Cl, 3-CN, 4-F and 3-CF_3, 3,4,5-trifluorophenyl significantly improved in vitro enzyme inhibitory activities (**13a**, **13f**). In case of methyl substituent in place of hydrogen in piperidine, 3-F (**15a**) and 3-Cl, 4-F (**15d**) displayed potent c-Met enzyme inhibitory activities and cell proliferation inhibitory activities. Introduction of hydroxyethyl

Table 1
SAR of substituent of R^1 and R^2 on pyridazinones[11]



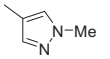
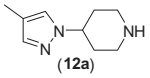
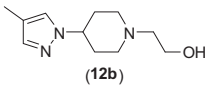
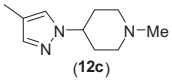
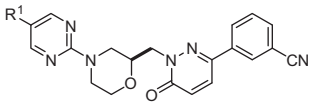
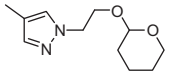
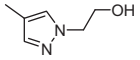
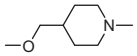
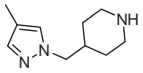
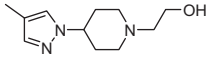
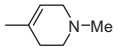
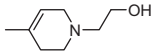
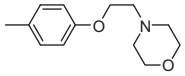
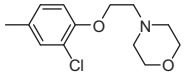
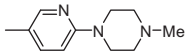
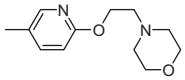
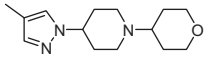
Entry	R^1	R^2	c-Met IC_{50} (μM)	Hs746T IC_{50} (μM)	H1993 IC_{50} (μM)	H3122 IC_{50} (μM)	U87MG IC_{50} (μM)
7a		4-OCH_2Ph	0.72	3.02	4.1	>10	7.6
13a	 (**12a**)	3-CN	0.005	0.0012	0.11	>10	>10
13b	**12a**	3,5-Difluoro	0.027	0.45	1.6	1.9	3.2
13c	**12a**	3-Cl	0.055	0.75	3.4	>10	9.9
13d	**12a**	3-Cl, 4-F	0.011	0.071	0.79	1.7	1.2
13e	**12a**	3-CF_3	0.021	0.084	1.3	1.4	2.5
13f	**12a**	3,4,5-Trifluoro	0.016	0.23	1.2	1.5	1.5
13g	**12a**	3-F	0.010	0.22	3.5	7.5	4.9
14a	 (**12b**)	3-F	0.007	0.46	3.2	3.3	3.4
14b	**12b**	4-F	0.020	0.28	3.8	>10	>10
14c	**12b**	3,4,5-Trifluoro	0.003	0.19		>10	4.07
15a	 (**12c**)	3-F	0.007	0.46	3.2	3.3	3.4
15b	**12c**	3,4,5-Trifluoro	0.019	0.48	3.6	7.6	9.1
15c	**12c**	4,5-Difluoro	0.021	0.94	5.7	>10	>10
15d	**12c**	3-Cl, 4-F	0.009	0.014	1.2	1.4	1.5

Table 2
SAR of substituent of R^1 on 3-cyanophenyl pyridazinones[11]



No	R^1	c-Met IC_{50} (μM)	Hs746T IC_{50} (μM)	H1993 IC_{50} (μM)	H3122 IC_{50} (μM)	U87MG IC_{50} (μM)
7b		0.004	0.0007	0.01	>1.0	>1.0
7c		0.021	0.46	2.6	>10	>10
7d		0.008	0.18	1.5	>10	>10
11		0.013	0.43	2.4	>10	>10
13h		0.006	0.039	0.68	>10	>10
14d		0.004	0.0034	2.1	>10	>10
16		0.040	0.74	3.8	>10	9.7
17		0.010	1.2	4.1	>10	>10
18		0.002	0.21	2.5	>10	>10
19a		0.028	0.41	1.8	>10	3.4
19b		0.090	1.42	1.2	>10	1.4
20a		0.11	7.146	7.4	>10	>10
20b		0.012	0.65	1.3	1.4	1.6
20c		0.035	0.74	3.7	9.8	nd
20d		0.005	0.013	0.23	>10	>10

moiety in place of methyl of piperidine displayed potent c-Met enzyme inhibitory activities and moderate cell proliferation inhibitory activities (**14a**, **14b**).

To understand the SAR of pyrimidine substituents at 5-position of pyrimidine, we investigated substituents with other functional groups, such as extended piperidines (**11**, **16**, **17**, **18**), pyrazoles (**7c**, **7d**, **13h**, **14d**, **20d**), phenyls (**19a**, **19b**), and pyridines (**20a**, **20b**, **20c**) in Table 2. Of the piperidine substituents, hydroxyethyl substituted tetrahydro-pyridine (**18**) exhibited highly potent in c-Met enzyme activity with moderate cell based activity in either Hs746T or H193 cell lines. Introduction of substituted pyrazoles exhibited potent enzyme inhibitory activities and cell base activity toward Hs746T and H1993 cell lines. In the initial assessment of the substituent effect of pyrazole, micromolar range of inhibitory activity was observed with methyl substituted pyrazole or R^1 and R^2 of benzyloxy substituted phenyl (**11**).

In particular, 4-tetrahydropyran substituted pyrazole (**20d**) displayed potent enzyme and cell based activities in both cell lines.

Molecular docking studies were performed using the Glide software contained in the Maestro 9.5 software with standard-precision (SP) options.[9] The crystal structure of c-Met (PDB code: 2RFS) was used with structural defects fixed by the Protein Preparation Wizard module.[10] The bound conformation of co-crystal ligand was generated with a good root mean square displacement (RMSD) of 0.2 A, showing the robustness of the docking protocols. Analysis of c-Met residues interacting with compounds reveals information valuable for the evaluation of binding affinity. The strong interaction of pyridazinones with c-Met is attributed to the strong hydrogen bonds between the pyrimidine moiety and the main chain NH of Met1160 hinge residue, and between pyridazinone and Asp1222. C2 atom of morpholine ring makes weak hydrogen bonding with Pro1158. In addition, hydrophobic interactions are also important for ligand binding. Pyrimidine ring positions at the narrow groove formed by Ile1084 and Met1160 of hinge region. And phenyl-pyridazinone group sandwiches between Met1211 and Tyr1230, making strong ring stacking interaction to Tyr1230. The potency of piperidine-containing compounds may be attributed to the hydrogen bonding with O atom of Val1083. The relative low binding affinity of compound **1** would be due to the lack of this hydrogen bonding. The 3-CN group may have

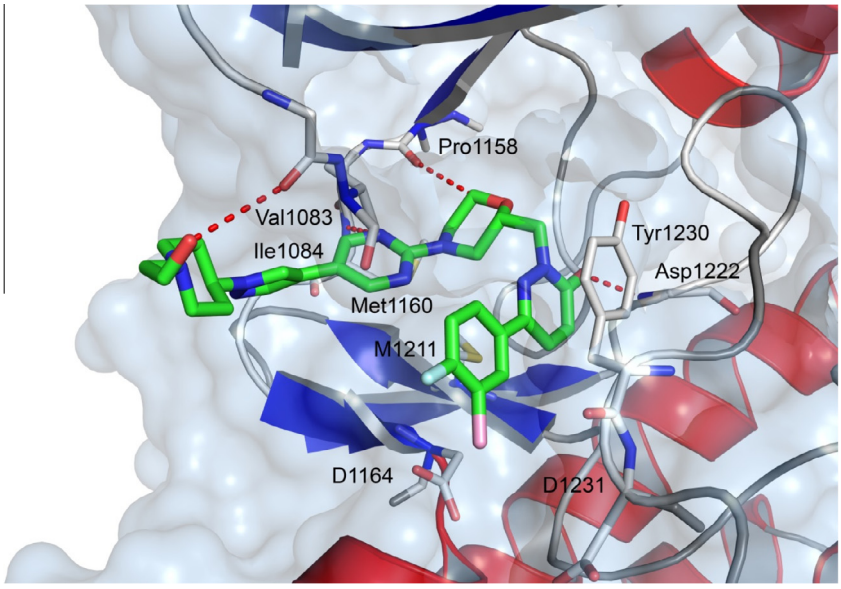


Figure 1. Binding model of compound **14a** in the c-Met pocket.

water-bridged ionic interactions with Asp1164 and Asp1231 (Fig. 1).

In summary, we have identified a series of pyridazinones with a substituted morpholine as potent c-Met kinase inhibitors. This series of compounds exhibited potent cell based activities in Hs746T and H1993 cell lines. Further studies for improving pharmacokinetic properties of pyridazinone derivatives in order to improve oral bioavailability and potent in vivo xenograft anti-tumor activity are underway in our research group.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.08.067.

References and notes

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11. *c-Met kinase assay*: Inhibition of kinase activity against recombinant c-Met protein was measured using homogeneous time-resolved fluorescence (HTRF) assays. Recombinant proteins containing c-Met kinase domain were purchased from Millipore. Optimal enzyme, ATP, and substrate concentrations were established using HTRF KinEASE kit (Cisbio) according to the manufacturer's instructions. Assays are composed of the c-Met enzyme mixed with serially diluted compounds and peptide substrates in a kinase reaction buffer (250 mM HEPES (pH 7.0), 0.5 mM orthovanadate, 0.05% BSA, 0.1% NaN3, 5 mM MgCl2, 1 mM DTT). Following the addition of reagents for detection, the Time Resolved-Fluorescence Resonance Energy Transfer (TRFRET) signal was measured using an EnVision multi-label reader (Perkin Elmer). Dose–response curves were generated to determine IC_{50} using Prism version 5.01 (GraphPad). Flt3 kinase assay was performed as described above using recombinant Flt3 protein in kinase reaction buffer (250 mM HEPES (pH 7.0), 0.5 mM orthovanadate, 0.05% BSA, 0.1% NaN3, 5 mM $MgCl_2$, 1 mM $MnCl_2$, 1 mM DTT). Reference compounds were included in each assay for plate uniformity (crizotinib) and select compounds were subjected to repeat experiments.*Proliferation assay*: Cells were plated in 96-well plates (10,000 cells per well) and serial dilutions of compounds were added. At the end of the incubation period (72 h), cell viability was measured by a tetrazolium dye assay using WST-8 [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2*H*-tetrazolium, monosodium salt] (Dojindo, Japan). IC_{50} was calculated by a nonlinear regression using Prism version 5.01.