Bioorganic & Medicinal Chemistry Letters 20 (2010) 1405–1409

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Discovery of 6-benzyloxyquinolines as c-Met selective kinase inhibitors

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ARTICLE INFO

Article history:
Received 4 November 2009
Revised 28 December 2009
Accepted 29 December 2009
Available online 4 January 2010

Keywords:
6-Benzyloxyquinolines
c-Met
Kinase inhibitor

ABSTRACT

A novel quinoline derivative that selectively inhibits c-Met kinase was identified. The molecular design is based on a result of the analysis of a PF-2341066 (**1**)/c-Met cocrystal structure (PDB code: 2wgj). The kinase selectivity of the derivatives is discussed from the view point of the sequence homology of the kinases, the key interactions found in X-ray cocrystal structures, and the structure–activity relationship (SAR) obtained in this work.

c-Met is a prototypic member of a subfamily of receptor tyrosine kinases (RTKs). c-Met is structurally distinct from other RTKs and is the only known high-affinity receptor for hepatocyte growth factor (HGF), also known as the scatter factor.[1,2] c-Met and HGF are dysregulated in human cancers and also contribute to dysregulation of cell proliferation, tumor invasion, and metastasis.[3,4] c-Met-activating point mutations in the kinase domain have been implicated as the cause of hereditary papillary renal carcinoma and also have been detected in sporadic papillary renal carcinoma and head and neck cancers.[5,6] Furthermore, amplification of the c-Met gene locus has been detected in patients with gastric and metastatic colorectal cancer.[7,8] These findings suggest that c-Met would be an attractive target for cancer therapy.

PF-2341066 (**1**) has been identified as an ATP-competitive c-Met inhibitor.[9] We analyzed the structure deposited in PDB (PDB code: 2wgj) and identified that the 2-aminopyridine bound to the hinge region of c-Met in a bidentate manner with the primary amine hydrogen-bonding to the carbonyl oxygen of P1158 and the pyridine nitrogen to the amide NH of M1160. Furthermore, we found that 2,6-di-chloro-3-fluorophenyl ring formed a π–π interaction with Y1230 at 0.40 nm, and a hydrophobic interaction with M1211 at 0.40 nm (Fig. 1). These hydrophobic interactions may contribute to the selective inhibition of c-Met since the two amino acids are conserved among only three (c-Met, AXL, MER) of the 491 kinases analyzed. In addition, GRID calculation (ver. 22a, Molecular Discovery Ltd, London, UK) revealed a hydrophobic space around the hinge region.

While exploring the scaffold of c-Met selective inhibitor, we hypothesized that the hydrophobic interactions were essential and the binding affinity from one of the hinge-interacting hydrogen bonds could be compensated by filling the hydrophobic space with the core structure. At the same time we found that no hydrogen bond was observed around the substituent that faced to the solvent-accessible region (green part in Fig. 1). We assumed that this moiety was primarily intended to adjust the physicochemical properties.[10] Based on these hypotheses, we designed core structure using molecular design software Moloc.[11]

We identified 6-benzyloxyquinoline as the scaffold to form the three interactions: (1) the hydrogen bond with the hinge region

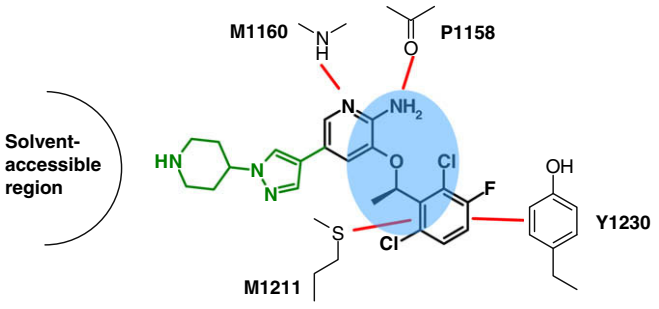

Figure 1. The interactions found in a PF-2341066 (**1**)/c-Met cocrystal structure and the hydrophobic space (cyan) identified by GRID analysis.

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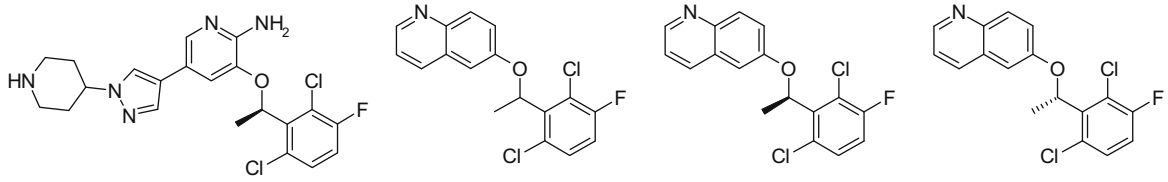
doi:10.1016/j.bmcl.2009.12.109

Table 1
Effects of configuration on inhibitory activity (IC_{50}/nM) against c-Met



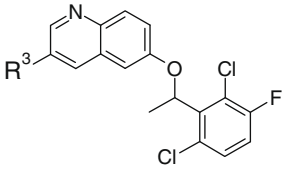
PF-2341066 (**1**) **2** (racemic) (*R*)-**2** (*S*)-**2**

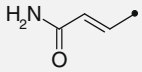
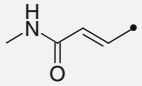
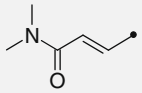
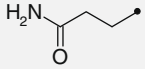
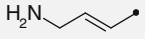
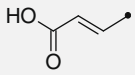
Compound	IC_{50} (nM)		
	c-Met	KDR	Src
1[a]	8	>10,000	Not available
(*R*)-**2**	130	23,000	22,000
(*S*)-**2**	1200	>50,000	20,000
2	453	32,000	>50,000

(M1160), (2) the hydrophobic and π–π interactions, and (3) hydrophobic space-filling with a quinoline skeleton. 6-Benzyloxyquinoline derivatives were prepared by treatment of commercially available 6-hydroxyquinoline with the corresponding alcohols prepared according to a patent procedure[12] under Mitsunobu conditions.[13] As a primary evaluation of kinase selectivity, we measured the inhibitory activities against kinase insert domain receptor (KDR) and Src kinase as a counter assay. The inhibition of KDR is reported to cause hypertension[14] and the inhibition of Src may link to the inhibition of other 10 kinases of Src family.[15] An optically active (*R*)-**2** showed inhibitory activity with a submicromolar IC_{50} value with high selectivity against KDR while its enantiomer (*S*)-**2** showed 10-fold less potent activity (Table 1). This result validates our hypotheses supporting the molecular design. Substitution at position 3 of the quinoline directed towards the solvent-accessible region of c-Met improved inhibitory activity. Thus, acryl amide substituted compound **3** (racemic) showed one order magnitude more potent c-Met inhibition retaining high selectivity than nonsubstituted compound **2** and potent cytotoxicity against a c-Met amplified gastric cancer cell line (MKN45). *N*-Monomethylated amide **4** had the similar kinase inhibitory activity and the cytotoxicity but *N*,*N*-dimethyl amide **5** showed weaker activities than **3**. Saturation of the double bond or removal of the carbonyl group of the acryl amide (compounds **6** and **7**, respectively) and the corresponding carboxylic acid **8** showed less inhibitory activities (Table 2).

A cocrystal of c-Met was obtained with **4** (a racemic compound, IC_{50} = 28 nM)[16] and its X-ray structure analysis revealed that *R*-enantiomer bound to the ATP binding site (M1160 backbone nitrogen–quinoline nitrogen = 0.28 nm) in a mode similar to that

Table 2
SAR for racemic quinoline derivatives



Compound	R^3	c-Met inh. (IC_{50}/nM)	KDR inh. (IC_{50}/nM)	Src inh. (IC_{50}/nM)	Cell growth inh. (MKN45) (IC_{50}/μM)
3		20	26,000	>50,000	0.35
4		28	>50,000	>50,000	0.24
5		59	>50,000	>50,000	0.61
6		205	50,000	>50,000	4.4
7		368	33,000	46,000	4.3
8		94	>50,000	>50,000	10.2

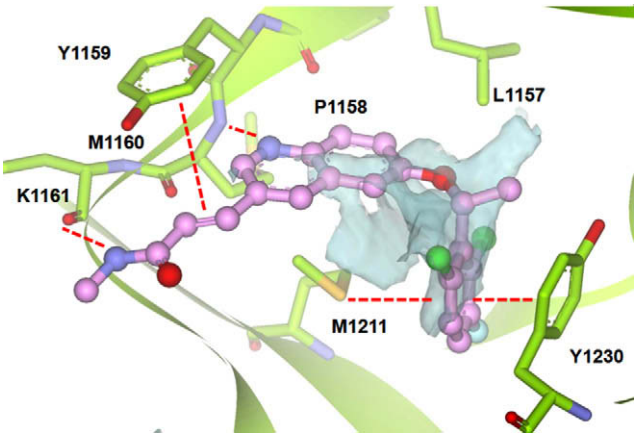
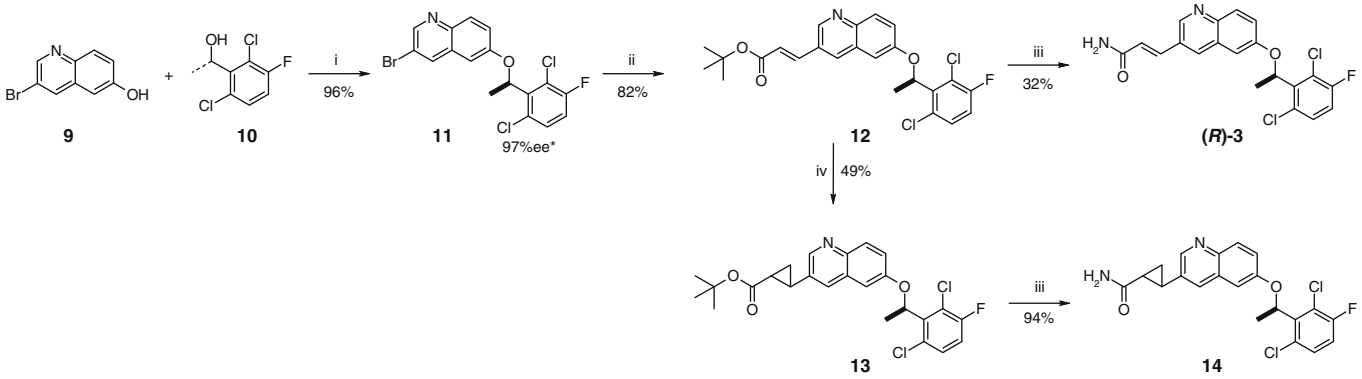


Figure 2. X-ray crystal structure of the c-Met (green)/compound (*R*)-**4** (pink) complex showing key interactions and the hydrophobic space (light blue) identified by GRID analysis.

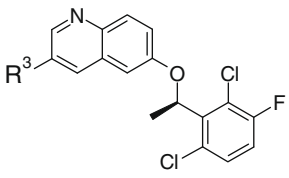
of **1** (Fig. 2). As expected, the hydrophobic side chain formed the hydrophobic interaction with M1211 (distance between sulfur atom and aromatic ring, 0.45 nm) in addition to the π–π interaction with Y1230 (distance between centers of the aromatic rings, 0.42 nm). The right ring of quinoline occupied the hydrophobic space found in the GRID analysis. The double bond of acryl amide formed a π–π interaction with Y1159 (distance between double bond and aromatic ring, 0.42 nm) and an amide hydrogen of **4** formed a hydrogen bond with K1161 (0.29 nm) but no interaction was found with the amide carbonyl oxygen of **4**. We assumed that the acryl amide moiety fixed the hydrogen donor at the appropriate position to form the hydrogen bond to K1161. These observations and assumptions correlate with the results showing the potency of saturated compound **6** and de-oxo compound **7** to be less than **3**. Because *N*,*N*-dimethyl acrylamide **5** has no NH group and carboxylic acid **8** is supposed to be deprotonated, these two compounds do not form hydrogen bond between the backbone carbonyl group of K1161. The contribution of the hydrogen bond to the inhibitory activity is small because the difference of inhibitory activity among compound **3**, **5** and **8** is not significant.

Although an acryl amide moiety is a good substituent in terms of kinase inhibitory activity, the drawbacks of **3** such as a low solubility and a potential risk of toxicity originated from Michael acceptor structure need to be eliminated. We designed a cyclopropyl moiety that avoids the Michael acceptor and maintains the position of the amide functional group. An optically active acrylamide (*R*)-**3** and cyclopropyl analog **14** were synthesized from known compound **9**[17] as shown in Scheme 1. Cyclopropanation of the acryl amide double bond was well tolerated, as compound **14** retained the kinase inhibitory activity and cytotoxicity against MKN45 and showed satisfactory solubility in fasted state simulated intestinal fluid (FaSSIF) (Table 3).



Scheme 1. Preparation of (*R*)-**3** and **14**. Reagents and conditions: (i) 1-(2,6-Dichloro-3-fluorophenyl)-ethanol, diisopropyl azodicarboxylate, PPh_3, THF, rt; (ii) *tert*-butyl acrylate, 10 mol % $Pd(OAc)_2$, tetrabutylammonium iodide, K_2CO_3, DMF, 100 °C; (iii) trifluoroacetic acid, CH_2Cl_2, rt and then water-soluble carbodiimide, hydroxybenzotriazole, diisopropylethylamine, DMF, rt; (iv) trimethylsulfoxonium iodide, NaH, DMSO, rt. * Chiral HPLC conditions: column, CHIRALPAK AD–H, 4.6 × 250 mm (Daicel); eluent, 4/1 0.1% diethylamine–EtOH/0.1% diethylamine–hexane; flow rate, 0.7 mL/min; column temp, 35 °C; detection, 334 nm. t_R = 8.0 min [(*R*)-isomer], t_R = 6.7 min [(*S*)-isomer].

Table 3
In vitro properties of compounds (*R*)-**3** and **14**



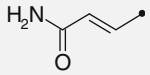
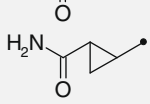
Compound	R^3	c-Met inh. (IC_{50}/nM)	Cell growth inh. (MKN45) (IC_{50}/μM)	Solubility in FaSSIF (μg/mL)
(*R*)-**3**		23	0.27	<13
14		9.3	0.093	345

Table 4
Inhibitory activity (IC_{50}/μM) of quinoline derivatives against various kinases

	(*R*)-**2**	(*R*)-**3**	**14**
Tyrosine kinase			
c-Met	0.13	0.023	0.0093
RON	3.6	0.45	0.23
AXL	14	0.57	0.43
EphA2	>50	4.4	5.2
KDR	>50	11	9.7
LTK	>50	18	10
FGFR2	24	6.9	12
Flt3	46	32	15
Fyn	>50	28	25
IGF1R	>50	>50	27
lck	>50	14	28
Kit	41	>50	41
EGFR	>50	>50	>50
PDGFR	>50	>50	>50
InsR	>50	>50	>50
YES	>50	26	>50
Src	>50	32	>50
BRK	>50	32	>50
Abl	>50	10	>50
Serine/threonine kinase			
AuroraA	>50	6.4	22
PKA	>50	>50	>50
AKT1	>50	>50	>50
cdk1	>50	>50	>50
cdk2	>50	>50	>50
PKC alpha	>50	>50	>50
PKC beta1	>50	>50	>50
PKC beta2	>50	>50	>50

Table 5
Growth factor effect on HUVEC anti-proliferative activity of compound **14**

Growth factor	HUVEC anti-proliferative activity (IC_{50}/μM)
HGF	0.3
VEGF	6.7
ECGS	9.9

Compound **14** showed the most potent inhibitory activity against c-Met among 19 tyrosine kinases[18] and 8 serine/threonine kinases[19] (IC_{50} ratios >20, Table 4) and was less cytotoxic against c-Met non-amplified 17 cell lines [colorectal cancer (HCT116, WiDr, COLO205), lung cancer (NCI-H460, A549, Calu-6), pancreatic cancer (AsPC-1, Capan-1, BxPC-3), prostate cancer (DU145, PC3, 22Rv1), breast cancer (MDA-MB-231, T47D, MCF7), gastric cancer (MKN28, NCI-N87)] (IC_{50} >1 μM) than MKN45.[20] The selective c-Met inhibition of **14** was properly reflected in an HGF-dependent anti-proliferative activity of HUVEC (Table 5).

Although a quinoline skeleton, as well as quinazoline and indolinone, is used as a scaffold in many kinases inhibitors,[21] the use of the 6-oxygen-functionalized quinoline scaffold is unique. This skeleton was designed to occupy the hydrophobic region adjacent to the hinge binding site found in the PF-2341066/c-Met cocrystal structure. The utility of this skeleton was demonstrated by the highly selective and potent c-Met inhibitor **14** which showed selective cytotoxicity reflecting selective c-Met inhibition.

Acknowledgments

The authors acknowledge H. Shirai for the enantiomeric excess determination, Dr. T. Matsuura and Dr. S. Kadono for protein production and its crystallization, and Y. Tachibana, K. Sakata and T. Fujii for their technical assistance with the kinase and cytotoxicity assays.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.12.109.

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12. Kung, P.-P.; Martinez, C. A.; Tao, J. Appl. Int. WO2006021885, 2006; *Chem. Abstr.* **2006**, *144*, 252788. Enantiomeric excess was determined by HPLC analysis: column, CHIRALPAK AD-H, 4.6 × 250 mm (Daicel); eluent, isopropyl alcohol/hexane = 5/95; flow rate, 1.0 mL/min; column temp, 35 °C; detection, 210 nm. The retention times were 6.6 and 6.1 min for (*R*)- and (*S*)-isomer, respectively.
13. The enantiomeric excess of the products was determined by chiral HPLC analysis. HPLC conditions: column, CHIRALPAK AD–H, 4.6 × 250 mm (Daicel); eluent, 0.1% diethylamine–EtOH; flow rate, 0.4 mL/min; column temp, 35 °C; detection, 210 nm.
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16. Cocrystals were obtained in 2 days under the crystallization conditions of 4 mg/ml protein, **5** (1 mM), 14% (w/v) PEG MME 5000, 12% (v/v) 2-methyl-2,4-pentanediol, 5% (v/v) 2-propanol and 0.1 M Tris–Cl (pH 7.5) with streak seeding, incubated at 12 °C. The structure was solved at 2.7 Å and the coordinates have been deposited in the Protein Data Bank, http://www.rcsb.org/pdb/home/home.do (PDB ID: 3a4p).
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18. The inhibitory activity against tyrosine kinase was measured by quantitative analysis of the phosphorylation of the substrate peptides by recombinant enzyme proteins in the presence of test articles using a europium-labeled antiphospho-substrate antibody (PerkinElmer). Five micro liter of solutions of compound, 10 μL of the substrate/ATP solution, and 5 μL of the enzyme solution were mixed sequentially and incubated for 90 min at 30 °C. The quantity of enzyme and ATP and the kind of substrate and cation species added in each assay are given in Supplementary Table 1. After the reaction was stopped by the addition of 10 μL of Stop solution, 20 μL of TR-FRET reagent (PerkinElmer) was added and incubated for 5 min at room temperature. After incubation, time-resolved fluorescence was measured by EnVison HTS (Model 2101, PerkinElmer, Inc.).
19. The inhibitory activity against various kinds of enzymes was measured by quantitative analysis of the phosphorylation of the substrate peptides by the recombinant enzyme proteins in the presence of test articles using an IMAP FP Screening Express Kit (Molecular Devices). Five micro liter of solutions of compound, 10 μL of the substrate/ATP solution, and 5 μL of the enzyme solution were mixed sequentially and incubated for 90 min at 30 °C. The

quantity of enzyme and ATP and the kind of substrate and cation species added in each assay are given in Supplementary Table 1. Sixty micro liter of IMAP reagent was added and the mixtures were incubated for 5 min at room temperature. After incubation, fluorescence polarization was measured by EnVision HTS.

20. All cancer cell lines were purchased from American Type Culture Collection. HUVEC were treated with compound in the presence of 20 ng/mL hepatocyte growth factor (HGF) (R&D systems), vascular endothelial growth factor (VEGF) (R&D systems), endothelial cell growth Supplement (ECGS), (BD Biosciences). Cells were treated with various concentrations of compound for 96 h. Cytotoxicity of compound was determined using a WST-8 cell counting kit (Dojin Laboratories).
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