Bioorganic & Medicinal Chemistry Letters 19 (2009) 397–400

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Discovery of a novel series of quinoxalines as inhibitors of c-Met kinase

John Porter *, Simon Lumb, Fabien Lecomte, James Reuberson, Anne Foley, Mark Calmiano, Kelly le Riche, Helen Edwards, Jean Delgado, Richard J. Franklin, Jose M. Gascon-Simorte, Alison Maloney, Christoph Meier, Mark Batchelor

UCB Celltech, 216 Bath Road, Slough SL1 3WE, United Kingdom

ARTICLE INFO

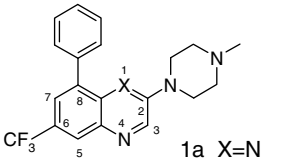
Article history:
Received 24 October 2008
Revised 17 November 2008
Accepted 18 November 2008
Available online 21 November 2008

Keywords:
Kinase Inhibitor
c-Met
Quinoxaline

ABSTRACT

A series of quinoxaline inhibitors of c-Met kinase is described. The postulated binding mode was confirmed by an X-ray crystal structure and optimisation of the series was performed on the basis of this structure. Future directions for development of the series are discussed together with the identification of a novel quinoline scaffold.

Members of the receptor tyrosine kinase (RTK) family are attractive targets for cancer therapy as inhibition can disrupt signaling pathways that mediate tumour formation and growth. c-Met kinase is a member of this family that, together with its ligand, hepatocyte growth factor (HGF), is important for normal mammalian development. However, c-Met has been shown to be deregulated and associated with high tumour grade and poor prognosis in a number of human cancers.[1] c-Met can become activated by a variety of mechanisms, including gene amplification and mutation inducing motility, invasiveness and tumourgenicity into the transformed cells.[2] Activation leads to receptor dimerisation and recruitment of several SH2 domain containing signal transducers that activate a number of pathways including the Raf-Mek-Erk and PI3k-Akt cascades. Targeting the ATP binding site of c-Met is a popular strategy for inhibition of the kinase, with a number of drug candidates reaching the clinical testing phase.[3] We now wish to report our efforts in this area.



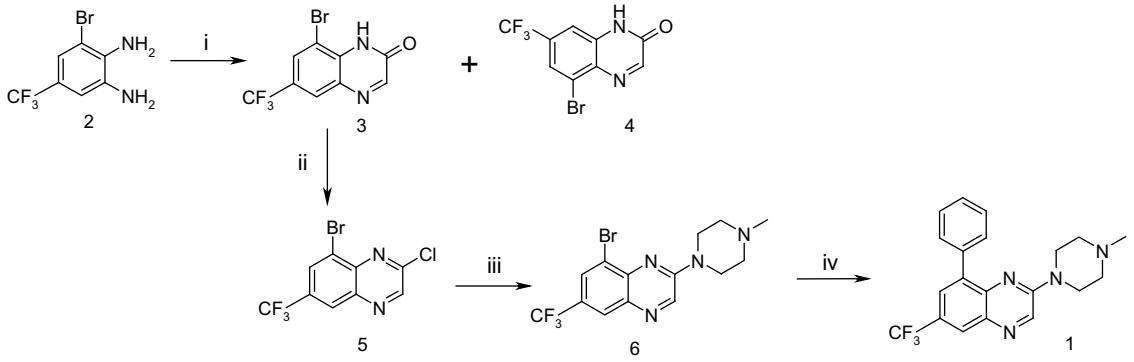
A HTS campaign identified the quinoxaline **1a** as a promising starting point. This low molecular weight, relatively soluble, ATP-competitive compound had an IC_{50} of 1.3 μM in the c-Met biochemical assay.[4] Cross-screening against a panel of over 200 kinases showed that **1a** was highly selective for c-Met.[5] Docking **1a** into the published crystal structure of c-Met (pdb:1R0P)[6] suggested that the N-4 atom of the quinoxaline ring formed a H-bond to Met1160 in the hinge region of the kinase, whilst the trifluoromethyl and phenyl groups occupied hydrophobic pockets, and the piperazine group was directed towards solvent. With this binding mode in mind we embarked on an exploration of the SAR.

The quinoxalines were prepared as shown in Scheme 1. Commercially available diamine **2** was cyclised to give a 1:1 mixture of regioisomeric quinoxalinones **3** and **4** from which the desired isomer **3** was isolated by trituration in 45% yield. Chlorination at the 2-position allowed the substitution of a range of amines and was followed by Suzuki coupling to give the desired products. Variation of substitution at the 6-position was achieved by appropriate choice of starting material. A related series of quinolines were prepared as shown in Scheme 2. Bromination, reduction and a Sandmeyer reaction[7] transformed the commercially available quinoline **7** to the quinolinol **10**. Ether formation under mildly basic conditions was followed by a Buchwald–Hartwig reaction[8] to give the quinoline **12**.

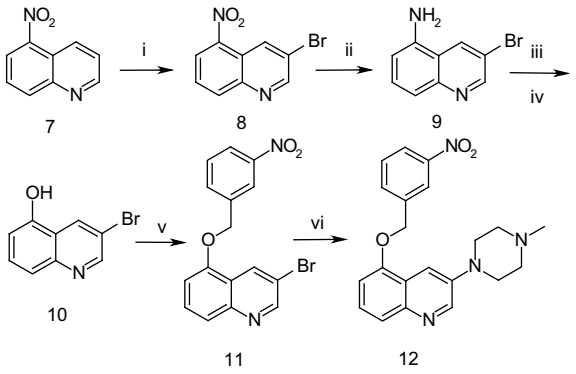
Replacement of the *N*-methyl piperazine with other groups showed that it is important to have a basic, preferably tertiary, nitrogen substituent at the quinoxaline 2-position, although the O-linked piperidine, **18**, retained some potency, Table 1. We postulate that the presence of an exocyclic heteroatom counteracts the electron withdrawing properties of the nitrogen atom at the 1-po-

* Corresponding author. Tel.: +44 1753 534655.
E-mail address: john.porter@ucb-group.com (J. Porter).

0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.11.062

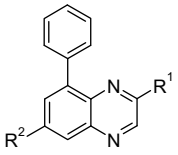


Scheme 1. Preparation of the quinoxalines. Reagents and conditions: (i) $HCOCO_2Et$, EtOH, reflux; (ii) $POCl_3$; (iii) *N*-methylpiperazine, THF, reflux; (iv) $PhB(OH)_2$, $Pd(PPh_3)_4$, dioxane, Na_2CO_3, reflux.



Scheme 2. Preparation of the quinolines. Reagents and conditions: (i) NBS, AcOH, reflux; (ii) Fe, $FeCl_3$, EtOH, AcOH, reflux; (iii) $NaNO_2$, AcOH, H_2SO_4, water, 0 °C; (iv) 10% aq H_2SO_4, reflux; (v) 3-nitrobenzylbromide, Cs_2CO_3, THF, 25 °C; (vi) *N*-methylpiperazine, $Pd_2(dba)_3$, BINAP, NaO*t*Bu, toluene, reflux.

Table 1
SAR of substituents in the 2- and 6-positions of 8-phenylquinoxaline



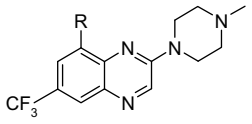
Compound	R^1	R^2	c-Met IC_{50} (μM)
1a	*N*-Methyl piperazinyl	CF_3	1.3
13	H	CF_3	0% @ 100 μM
14	Amino	CF_3	20.1
15	Dimethylamino	CF_3	5.3
16	Acetamido	CF_3	0% @ 100 μM
17	*N*-Acetyl piperazinyl	CF_3	1.3
18	*N*-Methyl piperidin-4-oxy	CF_3	19.6
19	*N*-Methyl piperazinyl	H	28.3
20	*N*-Methyl piperazinyl	F	3.9
21	*N*-Methyl piperazinyl	Cl	2.8
22	*N*-Methyl piperazinyl	Me	5.0
23	*N*-Methyl piperazinyl	CN	60.2

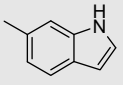
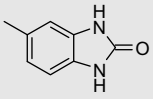
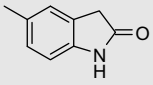
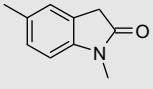
sition, strengthening the putative H-bond between N-4 and the hinge residue Met1160. This hypothesis was strengthened by the observation that the quinoline **1b** (IC_{50} 0.65 μM) was nearly twice as potent as **1a**. The trifluoromethyl group at the 6-position appears to be preferred, presumably optimally occupying a hydrophobic pocket that can also accommodate fluoro, **20**, chloro, **21**, or methyl, **22**, groups, though not for example, a nitrile group, **23**.

Realising that there was scope for optimisation of both potency and solubility by appropriate substitution at the 2-position, we now turned our attention to the 8-position where a study of the published X-ray crystal structures led us to believe that there was the greatest scope for enhancement of potency. A wide range of substituted phenyl analogues were prepared, a representative selection are shown in Table 2. Although bulky functionality was not accommodated, for example, **26**, most smaller substituents did not have any significant effects on potency. However, indolinones **33** and **34** and the benzimidazoline **32** did show promising activity

In an attempt to rationalise these observations, we solved the X-ray crystal structure of the c-Met-**28** complex[9], Figure 1a. Although the binding mode was revealed to be as we had predicted, the complex co-crystallised with a molecule of γ-butyrolactone

Table 2
SAR of substituents at the quinoxaline 8-position



Compound	R	c-Met IC_{50} (μM)
24	3,4-Dimethoxyphenyl	1.5
25	2,3-Dichlorophenyl	1.8
26	4-Benzyloxyphenyl	0% @ 100 μM
27	3-Carbamoylphenyl	5.0
28	3-Hydroxyphenyl	0.9
29	4-Hydroxyphenyl	0.8
30	3-Nitrophenyl	12.9
31		2.0
32		0.39
33		0.17
34		0.33

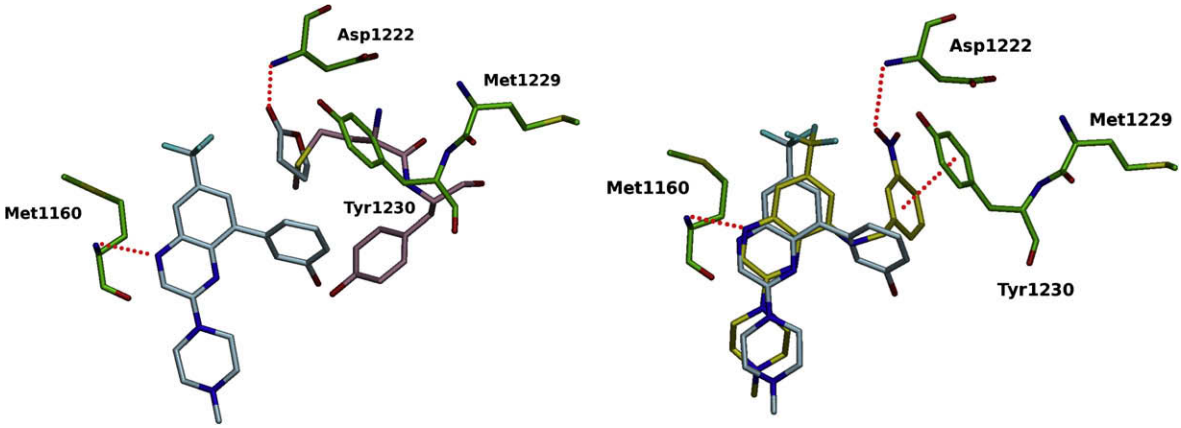
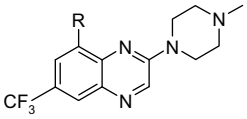
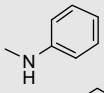
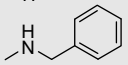
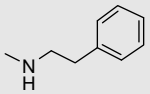
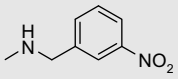
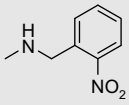
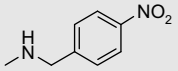
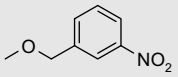
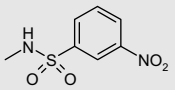
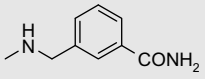
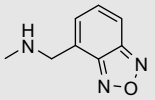
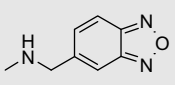


Figure 1. (a) X-ray crystal structure of the c-Met-**28**-γ-butyrolactone complex showing key interactions. The protein residues are in green with the positions of Met1229-Tyr1230 from PDB:1ROP shown in pink for comparison. (b) Overlay of a model of **38** (yellow) with the structure of **28** (grey) showing postulated interaction of the nitro group with Asp1222 and the phenyl ring with Tyr 1230.

Table 3
SAR of homologated substituents at the quinoxaline 8-position



Compound	Substituent	c-Met IC_{50} (μM)
35		1.9
36		1.1
37		7.1
38		0.035
39		0.54
40		0.055
41		0.32
42		0.017
43		0.73
44		0.031
45		0.38

from the buffer solution, which appeared to cause a conformational change to Met1229 and Tyr1230 (compared to pdb:1ROP[6]). This conformational change is similar to that observed in the crystal structure of SU11274 (pdb:2RFS) and has been suggested to be responsible for the difference in profiles of c-Met inhibitors.[10] The γ-butyrolactone also appeared to be forming a H-bond to the backbone NH of Asp1222. Consequently, we attempted to both incorporate this feature and overlap the π-cloud of the displaced Tyr1230 in our molecules by moving the aromatic 8-substituent further from the quinoxaline scaffold.

Incorporation of N- or O-linked substituents onto the bromide **6**, Scheme 1 using Buchwald–Hartwig chemistry[11] allowed us to prepare the desired compounds, some of which are outlined in Table 3. A two atom linking chain appears to be optimum, but the most significant observation is the effect of the 3-nitro substituent, **38**, which produces a 30-fold increase in potency. We believe that the electron withdrawing effect of the nitro group enhances π-stacking with the phenyl ring of Tyr1230 and forms a H-bond with Asp1222, Figure 1b. Compound **38** retained the selectivity profile of the parent **1a** in the kinase screening panel.[5] Interestingly, the analogous **30**, Table 2, was only weakly active. Replacing the aminomethyl linker with sulfonamide, for example **42**, improved potency still further. As the nitro group can confer mutagenic and carcinogenic properties[12] we attempted to identify isosteric groups to overcome these potential issues. The benzoxadiazole group has been reported as an isosteric replacement for the nitro group in a PDE4D inhibitor[13] and the calcium antagonist isradipine.[14] Incorporation of this moiety into our scaffold gave **44** whose equipotency with **38** suggests a similar mode of binding. However, the regioisomeric **45** was 10-fold less active than the corresponding 4-nitro analogue **40**.

Attempts to identify robust routes to the appropriately substituted 3,5,7-quinolines have proved unsuccessful; a survey of the literature suggests this is a relatively unexplored substitution pattern. However, we have prepared the analogous 3,5-disubstituted quinoline **12**, scheme 2, and were pleased to observe an improved potency in the biochemical assay (IC_{50} 0.057 μM) suggesting that 3,5,7-trisubstituted quinolines could form a series of c-Met inhibitors with a similar binding mode to the quinoxalines.

In conclusion we have identified novel quinoxaline and quinoline inhibitors of c-Met kinase and have rationalised the SAR by reference to an X-ray crystal structure.

References and notes

1. Birchmeier, C.; Birchmeier, W.; Gherardi, E.; van de Woude, G. F. *Nat. Rev. Mol. Cell Biol.* **2003**, *4*, 915.

2. (a) Christensen, J. G.; Burrows, J.; Salgia, R. *Cancer Lett.* **2005**, *225*, 1; (b) Ma, P. C.; Maulik, G.; Christensen, J.; Salgia, R. *Cancer Metastasis Rev.* **2003**, *22*, 309.
3. (a) Cui, J. J. *Expert Opin. Ther. Patents* **2007**, *17*, 1035; (b) Comoglio, S. G.; Giordano, S.; Trusolino, L. *Nat. Rev. Drug Disc.* **2008**, *7*, 504.
4. IC_{50} values for inhibitors of c-Met were determined using an IMAP time resolved fluorescence resonance energy transfer (TR-FRET) assay. 50 nM 6 His-tagged recombinant human c-Met residues 974-end (Millipore) was incubated in 20 mM Tris, 10 mM $MgCl_2$, 2.5 mM $MnCl_2$, 0.01% Tween 20 and 2 mM DTT with 5 μM ATP and 200 nM 5FAM-KKKSPGEYVNIGFG-NH2 in a total volume of 25 μl for 60 min at ambient temperature. Inhibitors were tested in duplicate at 10 concentrations starting from 20 μM at a final concentration of 1% DMSO. The reaction was stopped by addition of 50 μl of IMAP stop solution containing 60%Buffer A:40%Buffer B and a 1in 400 dilution of beads and Terbium reagent. Plates were read after an overnight incubation at +4 °C on an Analyst HT reader. Reported IC_{50}'s are from a minimum of two experiments ($n = 2$). Data analysis was carried out using a four parameter curve fit. The standard errors of the mean were calculated and expressed as a percentage of the mean IC_{50}. The average for this value was 12%.
5. Millipore Bioscience Division, Millipore UK Ltd, Gemini Crescent, Dundee Technology Park, Dundee, DD2 1SW, United Kingdom. http://www.millipore.com. Compound **1a** showed weak activity (<30% activity remaining @10 μM) against nine kinases including Flt family members and KDR. Similarly, compound **38** showed weak activity against 15 kinases, including Flts, Ron, Abl, Axl and TrkA.
6. Schiering, N.; Knapp, S.; Marconi, M.; Flocco, M. M.; Cui, J.; Perego, R.; Rusconi, L.; Cristiani, C. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 12654.
7. Hartley, C. S.; Elliott, E. L.; Moore, J. S. *J. Am. Chem. Soc.* **2007**, *129*, 4512.
8. Wolfe, J. P.; Buchwald, S. L. *J. Org. Chem.* **2000**, *65*, 1144.
9. Crystallisation and solution of structure performed by Proteros Biostructures GmbH, Am Klopferspitz 19, D-82152, Martinsried, Germany, http://www.proteros.com. Crystallographic data for the structure in this Letter have been deposited with the PDB (pdb code 3F66).
10. Bellon, S. F.; Kaplan-Lefko, P.; Yang, Y.; Zhang, Y.; Moriguchi, J.; Rex, K.; Johnson, C. W.; Rose, P. E.; Long, A. M.; O'Connor, A. B.; Gu, Y.; Coxon, A.; Kim, T.-S.; Tasker, A.; Burgess, T. L.; Dussault, I. *J. Biol. Chem.* **2008**, *283*, 2675.
11. (a) Wolfe, J. P.; Wagaw, S.; Marcoux, J.-F.; Buchwald, S. L. *Acc. Chem. Res.* **1998**, *31*, 805; (b) Shen, Q.-L.; Hartwig, J. F. *J. Am. Chem. Soc.* **2007**, *129*, 7734; (c) Surry, D. S.; Buchwald, S. L. *J. Am. Chem. Soc.* **2007**, *129*, 10354.
12. (a) Purohit, V.; Basu, A. K. *Chem. Res. Toxicol.* **2000**, *13*, 673; (b) Ashby, J.; Tennant, R. W. *Mutat. Res.* **1991**, *257*, 229.
13. Hersberger, R.; Dawson, J.; Meuller, T. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 233.
14. Man I'nt Veld, A. J. *Am. J. Hypertension* **1991**, *4*, 96S.