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Bioorganic & Medicinal Chemistry Letters 17 (2007) 6567–6571
Bioorganic & Medicinal Chemistry Letters

Hit generation and exploration: Imidazo[4,5-*b*]pyridine derivatives as inhibitors of Aurora kinases

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Received 8 August 2007; revised 21 September 2007; accepted 23 September 2007
Available online 22 October 2007

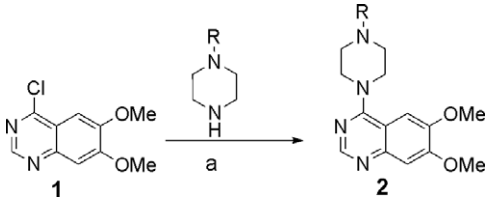
Abstract—A hit generation and exploration approach led to the discovery of **31** (2-(4-(6-chloro-2-(4-(dimethylamino)phenyl)-3*H*-imidazo[4,5-*b*]pyridin-7-yl)piperazin-1-yl)-*N*-(thiazol-2-yl)acetamide), a potent, novel inhibitor of Aurora-A, Aurora-B and Aurora-C kinases with IC_{50} values of 0.042, 0.198 and 0.227 μM, respectively. Compound **31** inhibits cell proliferation and has good microsomal stability.

The Aurora proteins A, B and C are serine/threonine kinases that play a key role in the regulation of mitosis, and are implicated in cancer initiation and progression.[1,2] Aurora-A and Aurora-B are overexpressed in a broad range of tumours[1,2] including breast,[3] colorectal,[4] testis,[5] ovarian[6] and glioma.[7] In addition, Aurora-A can transform cells when ectopically expressed in vitro.[4] The Aurora proteins have therefore emerged as attractive anticancer targets for the development of small molecule inhibitors as cancer therapeutic agents.

Several structurally diverse inhibitors of Aurora kinases with anti-tumour activity have been reported including quinazoline ZM447439,[8] VX-680[9] and the tetrahydropyrrolo[3,4-*c*]pyrazole PHA-680632.[10,11] More recently inhibitors selective for Aurora-A kinase (MLN8054[12]), and for Aurora-B (pyrazoloquinazoline AZD1152[13]), have been reported but it is not yet clear what is the ideal profile for therapeutic use. In this paper, we describe a novel class of imidazo[4,5-*b*]pyridines with activity against Aurora kinases.

Our main approach was based on high-throughput screening (HTS) of our in-house compound library against recombinant human Aurora-A kinase.[14] In parallel, we initiated a hit generation programme by producing a small, kinase-focused piperazinylquinazoline library via S_NAr substitution reactions on 4-chloro-6,7-dimethoxyquinazoline (Scheme 1 and Table 1). Members of this library (Table 1) displayed Aurora-A inhibitory activity, with compound **2f** being the most potent inhibitor (IC_{50} = 5.9 μM, Table 1). Despite the modest levels of inhibition observed, we were encouraged by the identification of the (piperazin-1-yl)-*N*-(thiazol-2-yl)acetamide substituent as a novel Aurora kinase inhibitor motif, which might be utilised in hit exploration.

From the HTS campaign the imidazo[4,5-*b*]pyridine **3** was identified (Fig. 1) as a hit. Compound **3** inhibited Aurora-A with an IC_{50} value of 0.57 μM, whilst its 6-H counterpart **4** (Fig. 1) was considerably less potent with an IC_{50} value of 4.3 μM, highlighting the 6-halo substituent as an important contributor to potency and an area of focus for further SAR work.



Scheme 1. Reagent and condition: (a) isopropyl alcohol, 105 °C, 7 h.

Keywords: Imidazo[4,5-*b*]pyridines; Aurora; Kinases.
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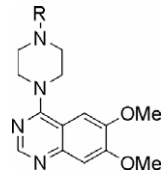
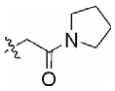
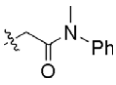
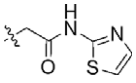
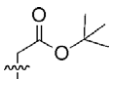
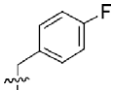
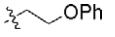
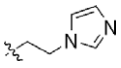
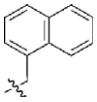
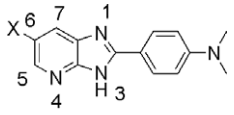
doi:10.1016/j.bmcl.2007.09.076

Table 1. Piperazinylquinazoline library: inhibition of Aurora-A



Compound	R	Aurora-A IC_{50} (μM)
2a		76
2b		na
2c		na
2d		98
2e		16.5
2f		5.9
2g		na
2h		40
2i		35
2k		na
2l		25
2m		9.6

Results shown are mean values for samples run in triplicate. The value for **2f** is the mean of two independent IC_{50} determinations. na, no activity at 100 μM.

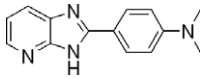
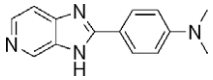
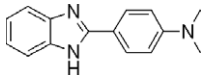
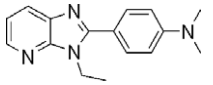


	X	IC_{50} (μM)
3	Cl	0.57 (±0.03)
4	H	4.3 (±0.29)

Figure 1. Inhibition of Aurora-A by compounds **3** and **4**.

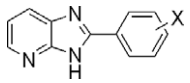
Access to **3**, **4** and compounds presented in Tables 2–5 was gained mainly by the oxidative condensation of 2,3-diaminopyridines with aldehydes in nitrobenzene

Table 2. Effect of N^4 and N^3–H on Aurora-A inhibition

Compound	Structure	Aurora-A IC_{50} (μM)
4		4.3 (±0.29)
7		12.5, 35% at 10 μM
8		8% at 100 μM
9		26% at 100 μM

Results shown are mean values for samples run in triplicate. The IC_{50} value for **4** is the mean of three independent IC_{50} determinations.

Table 3. Effect of substitution in aryl substituent



Compound	X	Aurora-A, IC_{50} (μM)
4	*p*-NMe_2	4.3 (±0.29)[a]
10	*m*-NMe_2	10.0[a]
11	*o*-NMe_2	20% at 10 μM[b]
12	*p*-OMe	6.6 (±3.18)[a]
13	*m*-OMe	16.0[a]
14	*o*-OMe	29[b]
15	*p*-Pyrrolidin-1-yl	4.6[a]
16	*p*-Pyrid-2-yl	4.4[a]
17	*p*-CN	38% at 10 μM[b]

[a] Mean of two independent IC_{50} determinations or mean (±SD) for $n > 2$.

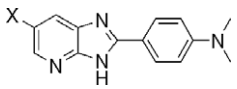
[b] Results are mean values for samples run in triplicate.

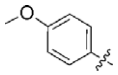
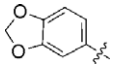
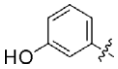
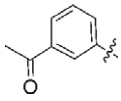
(Scheme 2).[15] Alternatively, compounds of this type (examples **10**, **11**, and **18**) could be produced by the cyclisation of 2,3-diaminopyridines with benzoic acids in $POCl_3$ (Scheme 2).[16] The N^3-ethyl derivative **9** (Table 2) was obtained by reacting 3-amino-2-ethylaminopyridine with 4-(dimethylamino)benzaldehyde in nitrobenzene. Compound **31** (Table 5) was prepared from the corresponding 2-amino-3-nitropyridine precursor and 4-(dimethylamino)benzaldehyde via a reductive cyclisation,[17] following a procedure reported by Yang et al.[18] This reductive cyclisation methodology was also applied to prepare compound **19** (Table 4) from 2-amino-3-nitro-5-trifluoromethylpyridine.[19]

Having confirmed the activity of **3** and **4**, we embarked on a hit-to-lead exploration programme focusing firstly on the importance of N^4 and N^3–H for inhibiting the enzyme, and then in turn the 2-aryl moiety, 6-substituent, 7-substituent and 6,7-combinations. Our aim was to explore SAR trends and identify lead series for further optimisation.

The importance of the pyridine N and the imidazole NH was investigated by preparing the imidazo[4,5-*c*]pyridine **7**, the benzimidazole analogue **8** and the *N*-ethyl deriva-

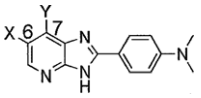
Table 4. Effect of substituent at 6-position

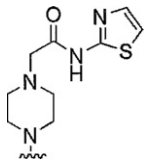
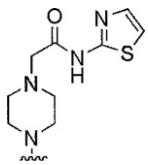


Compound	X (6-substituent)	Aurora-A IC_{50} (μM)
3	Cl	0.57 (±0.03)[a]
4	H	4.3 (±0.29)[a]
18	Br	0.49 (± 0.24)[a]
19	CF_3	0.74[a]
20	Me	6.9[a]
21		20[b]
22		33% at 100 μM[b]
23		18,[b] 31% at 10 μM[b]
24		9.0[a]
25		28% at 10 μM[b]
26		34% at 10 μM[b]

[a] Mean of two independent IC_{50} determinations or mean (±SD) for $n > 2$.
[b] Results are mean values for samples run in triplicate.

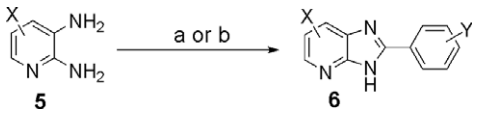
Table 5. Effect of substituent at 7-position, and 6,7-combinations



Compound	X	Y	Aurora-A IC_{50} (μM)
3	Cl	H	0.57 (±0.03)
4	H	H	4.3 (±0.29)
27	Cl	Cl	0.25
28	H	Me	7.0
29	H	Cl	2.4
30	H		0.87
31	Cl		0.042 (±0.022)

Values are mean of two independent IC_{50} determinations or mean (±SD) for $n > 2$.[24]

tive **9** (Table 2). The imidazo[4,5-*c*]pyridine derivative **7** was a slightly less potent inhibitor of Aurora-A than **4**, but compounds **8** and **9** were significantly less potent (Table 2) indicating that N^4 and N^3–H play an important role in binding to Aurora-A. A requirement for a hydrogen bonding interaction with the hinge region of the kinase is a plausible explanation, as this is commonly observed with ATP competitive inhibitors of kinases.[20] Docking studies based on the crystal structure of Aurora-A in complex with adenosine[21,22] confirmed that **3** can be accommodated at the ATP-binding site with bidentate H-bonds to Ala 213 in the hinge region of Aurora-A.



Scheme 2. Reagents and conditions: (a) aryl aldehyde, nitrobenzene, 150 °C, overnight; (b) $POCl_3$, aryl carboxylic acid, 120 °C, 6 h.

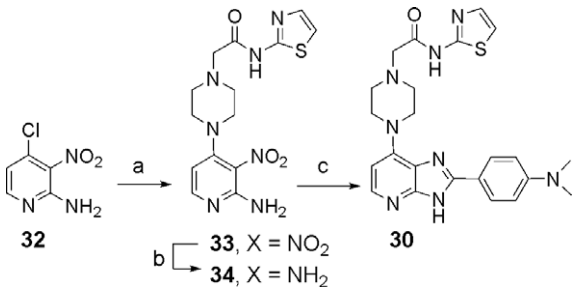
Investigation of the positional effect of the NMe_2 substituent revealed that the *para*-isomer (compound **4**) was a slightly more potent inhibitor of the enzyme than the *meta*-isomer **10**, and both are significantly more potent than the *ortho*-isomer **11**. A similar trend was observed with the OMe substituent (Table 3, compounds **12**–**14**). In addition to OMe, pyrrolidin-1-yl and pyrid-2-yl substituents were well tolerated (Table 3, compounds **12**, **15**, **16**).

The Aurora-A inhibitory effect of the 6-substituent was explored in more detail. The 6-Cl in **3** was replaced first by Br and CF_3 with retention of Aurora-A inhibitory activity (Table 4, compounds **18**, **19**). In contrast, the replacement of the 6-Cl in **3** by a Me group led to a loss of potency, and compound **20** inhibited Aurora-A with an IC_{50} value similar to that of the 6-unsubstituted analogue **4** (Table 4, compounds **4**, **20**). A 6-aryl substituent also had a detrimental effect on enzyme inhibition, with compounds **21**–**26** all being less potent than **3**, suggesting that any interaction at this position is size limited.

Access to the 6-aryl derivatives **21**–**26** was gained via a Suzuki cross-coupling reaction that worked well either under thermal conditions ($ArB(OH)_2$, $PdCl_2(dppf)$, DME, 1 M aq Na_2CO_3, 80 °C, 2.5–7 h) or microwave irradiation ($ArB(OH)_2$, $PdCl_2(dppf)$, DME, 1 M aq Na_2CO_3, 150 °C, 6 min) provided the N^3–H in **18** is protected. The MEM protecting group was used since it could be readily removed under acidic conditions (HCl, H_2O/THF).

The final part of this SAR exploration was focused on substitutions at the 7-position, and 6,7-combinations. The 7-Me derivative **28** had similar potency to the parent compound **4** (Table 5, compounds **4**, **28**). A marginal improvement in potency was seen with the 7-Cl substituted analogue **29** (Table 5, compounds **4**, **29**). Introduction of a 6-Cl substituent in **29** gave the 6,7-dichloro analogue **27** (Table 5)[23] that was approximately 10-fold more potent as an inhibitor of Aurora-A than **29**, a similar trend to that observed with compounds **3** and **4**.

Comparison of structure **4** and structure **2f** (a member of the piperazinylquinazoline library, Table 1) prompted



Scheme 3. Reagents and conditions: (a) 2-(piperazin-1-yl)-*N*-(thiazol-2-y)acetamide, isopropanol, 90 °C, 5 h; (b) EtOH/EtOAc, 10% Pd/C, 1 h; (c) 4-(dimethylamino)benzaldehyde, nitrobenzene, 140 °C, overnight.

the synthesis of the novel piperazinyl imidazo[4,5-*b*]pyridine derivative **30** (Table 5 and Scheme 3),[25] aiming at gaining potency from the additional interactions of the (piperazin-1-yl)-*N*-(thiazol-2-yl)acetamide moiety. Indeed, the incorporation of this fragment into **4** led to a ~5-fold potency improvement, compound **30** having an IC_{50} value of 0.87 μM (Table 5). Subsequent introduction of a 6-Cl substituent in **30** led to an additional 15-fold improvement in potency, and compound **31** had an IC_{50} = 0.042 μM. This gain in potency is similar to that observed earlier, when a 6-Cl substituent was introduced into structures **4** and **29** (see compounds **3**, **4** and **27**, **29**; Table 5).

In vitro kinase assays using purified recombinant proteins, showed that **31** inhibited Aurora-B and Aurora-C with IC_{50} values of 0.198 ± 0.050 and 0.227 ± 0.064 μM, respectively. Compound **31** was shown to inhibit HCT116 cell growth with a GI_{50} of 0.350 μM, and HeLa cell growth with a GI_{50} of 0.200 μM. It also displayed good metabolic stability with 75% of **31** remaining after a 30-min incubation with mouse liver microsomes. Regarding inhibition of cytochrome P450 isoforms, **31** showed $IC_{50} > 10$ μM for CYP1A2, CYP2A6, CYP2C19, CYP2D6 and ~10 μM for CYP3A4 and CYP2C9.[26,27]

In summary, two distinct chemical series of Aurora-A inhibitors were discovered; namely the imidazo[4,5-*b*]pyridines via HTS and quinazolines carrying N-substituted piperazines. SAR studies were undertaken and led to the hybrid structure **31** which is a potent, novel inhibitor of Aurora-A, Aurora-B and Aurora-C kinases with IC_{50} values of 0.042, 0.198 and 0.227 μM, respectively. In addition, **31** blocks proliferation of HCT116 colon cancer cells, (GI_{50} = 0.350 μM), has good microsomal stability and comparatively weak inhibitory activity versus cytochrome P450 isoforms in vitro, a balance of properties that led to the adoption of **31** as a lead compound for further optimisation studies.

Acknowledgments

The authors are grateful to Chroma Therapeutics, and in particular Dr. A. Davidson, Dr. A. Drummond and Dr. D. Moffat for valuable discussions. The work of the Cancer Research UK Centre for Cancer Therapeutics is funded primarily by Cancer Research UK [CUK] Grant C309/A2187. We thank F. Urban, Dr. N. E. Wilsher and Dr. F. Raynaud for metabolism and cytochrome P450 isoform experiments. Paul Workman is a Cancer Research UK Life Fellow. We also thank Dr. A. Mirza, A. Hayes and M. Richards for assistance with compound characterisation.

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25. 2-Amino-4-chloro-3-nitropyridine was prepared from 2-amino-4-chloropyridine by nitration (concd H_2SO_4, 70% HNO_3).
26. Inhibition of human liver CYP isozymes was assessed in human liver microsomes (pool of 50 individuals) as described in Ref. 27 with the following modifications: microsomal protein concentration 0.5 mg/ml, incubation time 10 min, mephenytoin as the CYP2C19 substrate and metabolite detection by LC–MS–MS ESI+ on a Shimadzu LC system connected to a QTRAP 4000 (Applied Biosystems).
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