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Structure-based design of imidazo[1,2-*a*]pyrazine derivatives as selective inhibitors of Aurora-A kinase in cells

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ABSTRACT

Co-crystallisation of the imidazo[1,2-*a*]pyrazine derivative **15** (3-chloro-*N*-(4-morpholinophenyl)-6-(pyridin-3-yl)imidazo[1,2-*a*]pyrazin-8-amine) with Aurora-A provided an insight into the interactions of this class of compound with Aurora kinases. This led to the design and synthesis of potent Aurora-A inhibitors demonstrating up to 70-fold selectivity in cell-based Aurora kinase pharmacodynamic biomarker assays.

The Aurora proteins A, B, and C belong to a small family of highly homologous serine/threonine kinases that play a critical role in regulating mitosis.[1–3] Aurora-A has been reported to be overexpressed in a wide range of tumours such as breast, colorectal, ovarian, and glioma.[4–7] Likewise, Aurora-B is overexpressed in human tumours including glioma, thyroid carcinoma, seminoma, and colorectal cancer.[8–10] During the last decade, the discovery of small-molecule inhibitors of Aurora kinases as potential anticancer agents has been the goal of numerous research groups,[11,12] and has resulted in a number of compounds (e.g., VX-680 (MK-0457),[13] PHA-739358,[14,15] AT9283,[16] SNS-314,[17] MLN8054,[18] and AZD1152[19]) being assessed in clinical trials. Herein, we report the discovery of imidazo[1,2-*a*]pyrazine derivatives as inhibitors of Aurora kinases, and the structure-based design of potent inhibitors demonstrating isoform selectivity for Aurora-A kinase in cell based pharmacodynamic biomarker assays.

High-throughput screening (HTS) of our in-house compound collection versus Aurora-A provided the imidazopyrazine derivatives **1** and **2** as promising hits (Fig. 1).[20] Having confirmed the Aurora-A inhibitory activity of **1** and **2** as 6.70 and 2.12 μM,

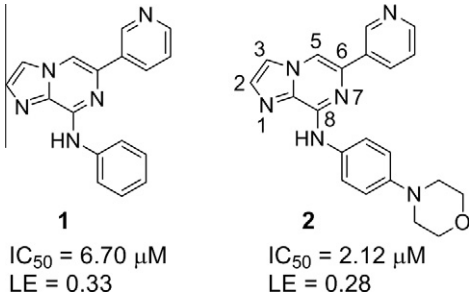
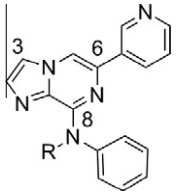


Figure 1. HTS hits, inhibition of Aurora-A.

Table 1
Effect of C8–NH and C8–NMe on Aurora-A inhibition



Compound	R	Aurora-A IC_{50} (μM)
1	H	6.70 ± 2.46
16	Me	27% Inhibition at 10 μM[a], 64.0[a]

Results shown are mean values of two independent determinations or mean (±SD) for n >2 unless specified otherwise.
[a] Results shown are mean values for samples run in triplicate.

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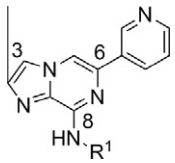
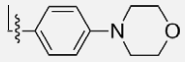
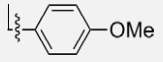
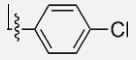
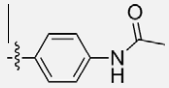
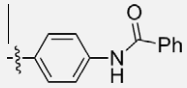
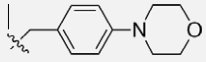
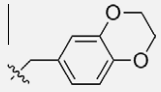
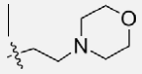
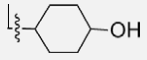
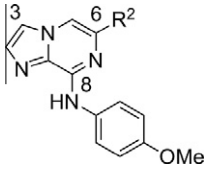
Table 2
Effect of C8-substituent on Aurora-A inhibition

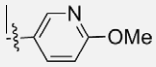
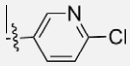
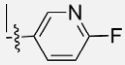
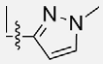
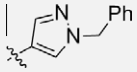
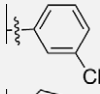
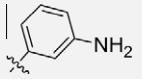
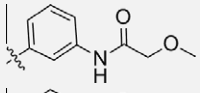
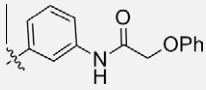
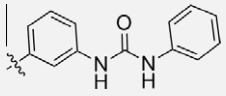
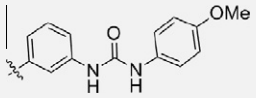
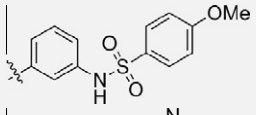
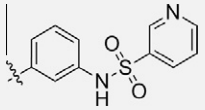


Compound	R^1	Aurora-A IC_{50} (μM)	Ligand efficiency[b]
1		6.70 ± 2.46	0.33
2		2.12 ± 0.63	0.28
6a		2.90	0.32
6b		5.5[a]	0.32
6c		2.5[a]	0.30
6d		3.75	0.24
6e		41% inhibition at 10 μM[a]	n.d.[c]
6f		35% inhibition at 10 μM[a]	n.d.[c]
6g		33% inhibition at 10 μM[a]	n.d.[c]
6h		32% inhibition at 10 μM[a]	n.d.[c]

Results shown are mean values of two independent determinations or mean (±SD) for n >2 unless specified otherwise.
[a] Results shown are mean values for samples run in triplicate.
[b] Calculated using the formula: LE = [−1.4 × $\log_{10}$ (IC_{50} (M))]/(number of heavy atoms).
[c] n.d. = not determined.

Table 3
Effect of C6-substituent on Aurora-A inhibition

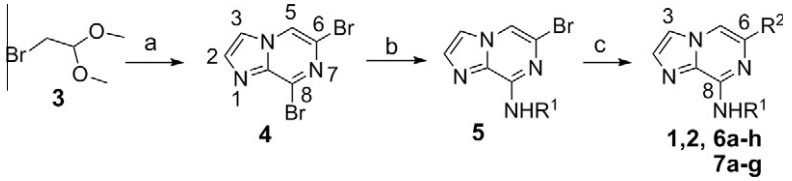


Compound	R^2	Aurora-A IC_{50} (μM)	Ligand efficiency[b]
6a		2.90	0.32
7a		36%, 30% inhibition at 10 μM[a]	n.d.[c]
7b		38%, 28% inhibition at 10 μM[a]	n.d.[c]
7c		42%, 32% inhibition at 10 μM[a]	n.d.[c]
7d		9.70[a]	0.29
7e		41%, 38% inhibition at 10 μM[a]	n.d.[c]
7f		46% inhibition at 10 μM[a]	n.d.[c]
7g		5.48 ± 2.37	0.29
7h		7.31 ± 3.35	0.24
7i		48%, 22% inhibition at 10 μM [a]	n.d.[c]
7j		7.20[a]	0.21
7k		7.90[a]	0.20
7l		6.70	0.20
7m		4.50	0.22

Results shown are mean values of two independent determinations or mean (±SD) for n >2 unless specified otherwise.
[a] Results shown are mean values for samples run in triplicate.
[b] Calculated using the formula: LE = [−1.4 × $\log_{10}$ (IC_{50} (M))]/(number of heavy atoms).
[c] n.d. = not determined.

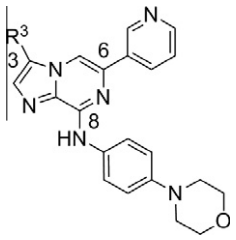
respectively, we initiated a hit-to-lead programme aimed at establishing clear structure–activity relationship (SAR) trends in relation to enzyme inhibition and identifying a compound series meriting further optimisation towards a preclinical development candidate. To achieve these goals, we first attempted to establish the role of the C8–NH of the imidazo[1,2-*a*]pyrazine template for enzyme inhibition. Subsequently, we investigated the Aurora-A inhibitory effect of C6, C8 and C3 substituents on the scaffold (Fig. 1).

Starting from 6,8-dibromoimidazo[1,2-*a*]pyrazine (**4**) which was synthesised by modifying a previously reported preparation,[21] access was readily gained to 6,8-disubstituted derivatives **1** and **2** (Fig. 1), **6a–h** (Table 2) and **7a–g** (Table 3), by first introducing the amino group at C8 via a S_NAr substitution reaction[22] followed by



Scheme 1. Reagents and conditions: (a) (i) 48% aq HBr, H_2O, 90 °C, 1 h, then cool to rt, (ii) $NaHCO_3$, ^{i}PrOH, 2-amino-3,5-dibromopyrazine, 90 °C, 5 h; (b) $ArNH_2$, $^i Pr_2NEt$, NMP, microwave, 190 °C, 30 min or benzyl/aliphatic amine, $^i Pr_2NEt$, BuOH, microwave, 110 °C, 30 min; (c) $R^2B(OH)_2$, $Pd(PPh_3)_2Cl_2$, 2 M aq Na_2CO_3, CH_3CN, microwave, 150 °C, 15–30 min; for compounds **7a–g**: $R^2B(OH)_2$/pinacol ester, $Pd(dppf)Cl_2$, 2 M aq Na_2CO_3, CH_3CN, microwave, 150 °C, 20 min.

Table 4
Effect of C3-substituent on Aurora-A and cell growth inhibition



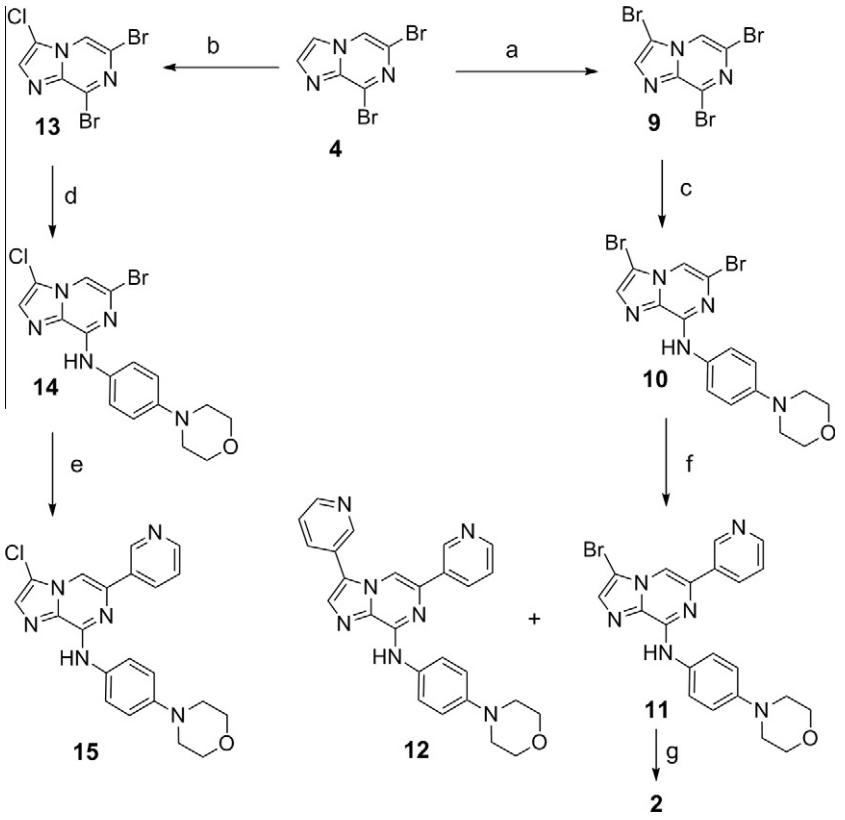
Compound	R^3	Aurora-A IC_{50} (μM)	Ligand efficiency[a]	HCT116 GI_{50} (μM)	Hela GI_{50} (μM)
2	H	2.12 ± 0.63	0.28	n.d[b]	n.d[b]
8	Me	0.208 ± 0.052	0.32	7.2	6.5
11	Br	0.152 ± 0.090	0.33	2.3	13.4
12		4.20	0.22	n.d[b]	n.d[b]
15	Cl	0.190 ± 0.138	0.32	3.9	28

Aurora-A IC_{50} results are mean values of two independent determinations or mean (±SD) for $n > 2$.
[a] Calculated using the formula: LE = $[-1.4 \times \log_{10}(IC_{50}\ (M))]$/(number of heavy atoms).
[b] n.d = not determined.

C6–C bond formation under standard Suzuki cross-coupling conditions (Scheme 1). The amide derivatives **7h**, **7i** (Table 3) were obtained from the aniline **7g** by reaction with the requisite acid chloride in the presence of diisopropylethylamine. The ureas **7j** and **7k** (Table 3) were synthesised by reacting the aniline **7g** with the appropriate aryl isocyanate in $CHCl_3$. The sulfonamides **7l** and **7m** (Table 3) were prepared by treating the aniline **7g** with 4-methoxybenzenesulfonyl chloride and pyridine-3-sulfonyl chloride, respectively, in pyridine/$CHCl_3$ (v/v; 1:1).

The 3-methylimidazo[1,2-*a*]pyrazine derivative **8** (Table 4) was accessed via the route shown in Scheme 1 by replacing **4** with 6,8-dibromo-3-methylimidazo[1,2-*a*]pyrazine which was prepared from 2-amino-3,5-dibromopyrazine in a manner similar to that reported for the synthesis of 8-bromo-6-chloro-3-methylimidazo[1,2-*a*]pyrazine.[23] The syntheses of the 3-bromo-, 3-chloro-, and 3-(pyrid-3-yl)-imidazo[1,2-*a*]pyrazine-based analogues (compounds **11**, **15**, **12**) are shown in Scheme 2. 3,6,8-Tribromoimidazo[1,2-*a*]pyrazine (compound **9**, Scheme 2) was obtained from 6,8-dibromoimidazo[1,2-*a*]pyrazine (**4**) upon treatment with NBS.[21,24] Access to 6,8-dibromo-3-chloroimidazo[1,2-*a*]pyrazine (compound **13**, Scheme 2) was accomplished in a similar manner to **9** but using NCS.[23,25,26] A Suzuki cross-coupling between **10** and 3-pyridylboronic acid pinacol ester gave the 3-Br derivative **11** in 41% yield, and the 3-(pyrid-3-yl) analogue **12** in 22% yield (Scheme 2). The assignment of regiochemistry in **11** was confirmed by a Pd-catalysed debromination of **11** with triethylsilane (Scheme 2).[27,28] The product of this reaction had a ^1H NMR identical to that of a sample of **2** obtained via the route shown in Scheme 1.

In an effort to establish the importance of C8–NH for enzyme binding, the C8-*N*-methyl derivative of **1** (compound **16**, Table 1) was prepared via the route shown in Scheme 1. Compound **16** displayed reduced Aurora-A inhibitory potency relative to **1**, indicating that C8–NH plays a role in binding to Aurora-A, a plausible explanation being a hydrogen bonding interaction with the hinge region of the kinase. Docking studies based on the crystal structure of Aurora-A in complex with adenosine[29] suggested that compound **2** could occupy the ATP binding site with the N1 of the imidazo[1,2-*a*]pyrazine core and C8–NH forming hydrogen bonding interactions with Ala213 in the hinge region of the kinase.



Scheme 2. Reagents and conditions: (a) NBS, CH_3CN/CH_2Cl_2, rt, 6 h; (b) NCS, CH_3CN/DCE (v/v; 3:1), reflux; (c) 4-(4-morpholino)aniline, $^iPr_2NEt$, dioxane/DMF (v/v; 8:1), microwave, 180 °C, 30 min; (d) 4-(4-morpholino)aniline, $^iPr_2NEt$, dioxane, microwave, 180 °C, 30 min; (e) 3-pyridylboronic acid, $Pd(PPh_3)_2Cl_2$, 2 M aq Na_2CO_3, CH_3CN, microwave, 150 °C, 30 min; (f) 3-pyridylboronic acid pinacol ester, $Pd(dppf)Cl_2$, 2 M aq Na_2CO_3, CH_3CN, microwave, 150 °C, 25 min; (g) 10% Pd–C, Et_3SiH, EtOH.

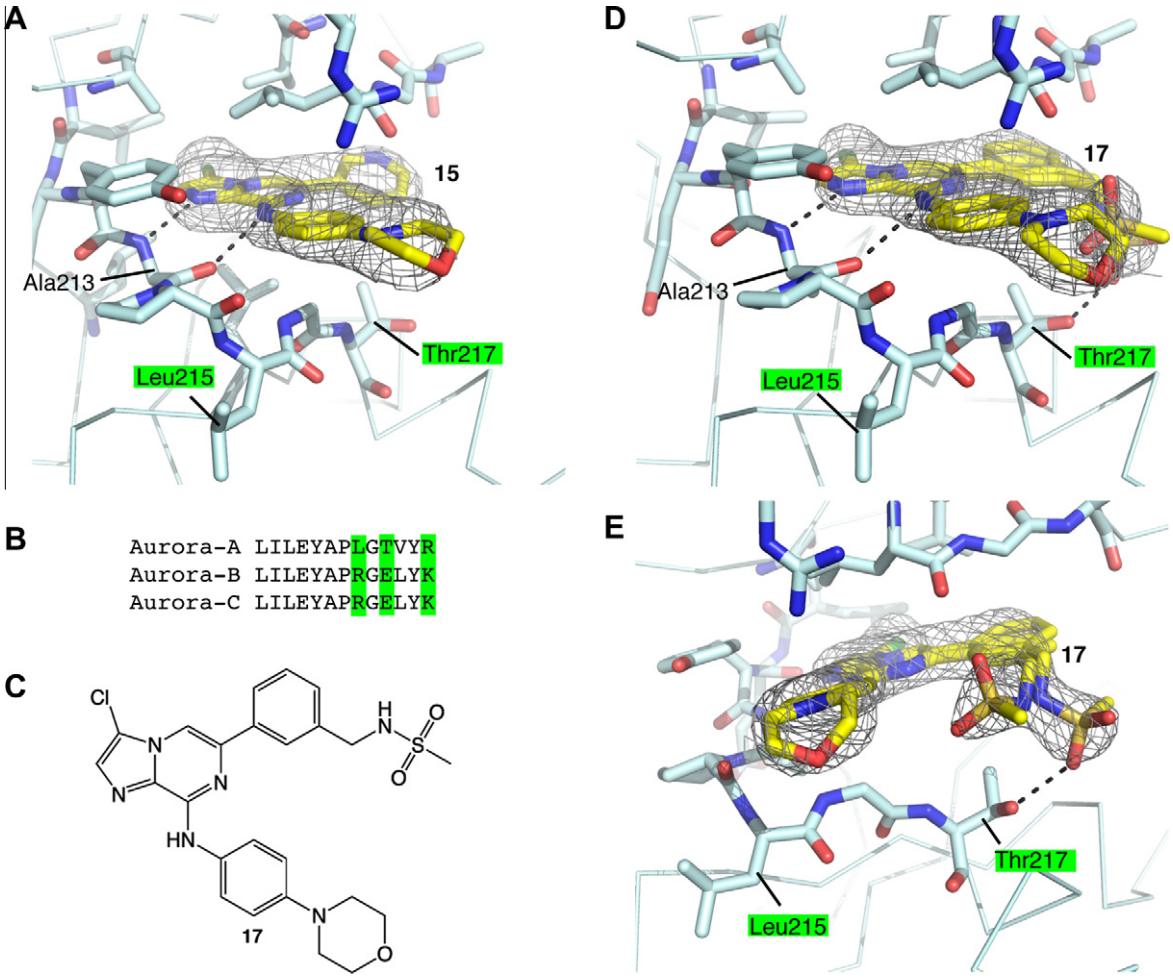


Figure 2. Co-crystal structures of compounds **15** and **17** bound to Aurora-A. A standard colour scheme is used throughout the figure: nitrogen, blue; oxygen, red; sulfur, orange; chlorine, green; protein carbon atoms, light blue; compound carbon atoms, yellow. Putative hydrogen bonds are shown as dashed, black lines. Residues that differ between Aurora-A and Aurora-B/C are highlighted in green. (A) Co-crystal structure of compound **15** bound to the catalytic domain of D274 N mutant of Aurora-A (PDB code 2XNE). (B) Sequence alignment of the hinge region of human Auroras -A, -B and -C shows the three residues which differ. (C) Chemical structure of compound **17**. (D) Co-crystal structure of compound **17** bound to the catalytic domain of wild-type Aurora-A (PDB code 2XNG). (E) Co-crystal structure of **17**/Aurora-A shown in a view rotated by 45° around the vertical axis with respect to that shown in (D). In this view, the interaction between Thr217 and one of the two conformations of **17** is clearly shown.

Subsequently, working on the hypothesis that the imidazo[1,2-*a*]pyrazine N1 and C8–NH bind to the hinge region of the protein, we investigated the Aurora-A inhibitory effect of the C6, C8, and finally the C3-substituent (Fig. 1).

We began by exploring substitution at the C8-position in an attempt to improve enzyme inhibitory activity. Compound **2** was a slightly more potent inhibitor of Aurora-A kinase compared with **1** (Fig. 1, Table 2), prompting us to introduce a range of *p*-substituents on C8–NHPh of **1** (compounds **6a–d**, Table 2). As shown in Table 2, analogues **6a–d** exhibited similar Aurora-A inhibitory potencies to that of the unsubstituted parent compound **1**. The IC_{50} values[30] varied between 2.5 and 5.5 μM with the morpholino, methoxy, and $NHCOCH_3$ derivatives being the most potent inhibitors of the enzyme. The introduction of a substituted benzyl (compounds **6e, 6f**; Table 2) or a substituted alkyl (**6g, 6h**, Table 2) had a detrimental effect on inhibitor potency. The data in Table 2 pointed to phenyl, 4-(morpholin-4-yl)phenyl and 4-methoxyphenyl as the preferred C8-substituents.

Subsequently, the effect of the C6-substituent on Aurora-A inhibition was explored in more detail. Replacement of the pyrid-3-yl in **6a** with a substituted pyrid-3-yl group (compounds **7a–c**, Table 3), 1-methyl-1*H*-pyrazole (compound **7d**), 1-benzyl-1*H*-pyrazole (compound **7e**), and 3-chlorophenyl (compound **7f**) provided no Aurora-A inhibitory benefit (Table 3). However, the introduction of 3-aminophenyl as a C6-substituent was better tolerated; compound **7g** displayed comparable potency (IC_{50} = 5.48 μM) to that of **6a** (Table 3). Subsequent utilisation of NH_2 in **7g** as a tether for additional functionalisation provided the amides **7h**, **7i**, ureas **7j**, **7k** and sulfonamides **7l**, **7m**; all but **7i** inhibited Aurora-A kinase with IC_{50} values similar to that of **7g**. Overall, the data in Table 3 pointed to the pyrid-3-yl as the optimum, most ligand efficient,[31] C6-substituent.

The final part of this exploration was focussed at the C3-position of the imidazo[1,2-*a*]pyrazine scaffold, an area of investigation that proved more promising. Introduction of a 3-Me group in **2** provided a significant benefit on Aurora-A inhibition. Compound **8** was an approximately 10-fold more potent inhibitor of Aurora-A compared with **2** (Table 4). A similar trend was observed with the C3-bromo and C3-chloro analogues (compounds **11** and **15**, Table 4) that inhibited Aurora-A with IC_{50} values of 0.152 and 0.190 μM, respectively. In contrast, the introduction of a C3-pyrid-3-yl substituent in **2** (compound **12**, Table 4) led to a slight drop in potency (Aurora-A IC_{50} = 4.20 μM, Table 4). In relation to Aurora isoform selectivity, compound **15** inhibited recombinant human Aurora-C with an IC_{50} value of 1.95 μM,[32] less potently compared with Aurora-A. Aurora isoform selectivity was also determined in a cell-based assay in which the autophosphorylation of T288 in Aurora-A was used as a biomarker for Aurora-A inhibition and

the phosphorylation of histone H3 at S10 as a biomarker for Aurora-B inhibition.[18] The cellular Aurora-A and Aurora-B IC_{50} values for **15** were determined as 0.381 and 7.48 μM, respectively, indicating an approximately 19-fold selectivity in inhibiting Aurora-A.

Encouraged by the significant increase in Aurora-A inhibitory potency with analogues **8**, **11**, and **15**, we studied their cell growth inhibitory activity in two cancer cell lines (Table 4). All three compounds displayed cellular potency with GI_{50} values between 2.3 and 28 μM (Table 4).[33]

The binding mode of this class of imidazo[1,2-*a*]pyrazine-based kinase inhibitors was elucidated by co-crystallisation of Aurora-A catalytic domain D274N mutant with **15** to a resolution of 2.8 Å (Fig. 2A, Supplementary data Table S1).[34] As we hypothesised, **15** occupies the ATP-binding site with the imidazo[1,2-*a*]pyrazine N1 and C8–NH forming hydrogen bonding interactions with the Ala213 in the hinge region of the kinase. Imidazo[1,2-*a*]pyrazine N1 is hydrogen bonded to the main chain NH of Ala213 (3.0 Å) and the C8–NH to the carbonyl of Ala213 (2.8 Å) as shown in Figure 2A. The chlorine atom of the inhibitor sits in a hydrophobic pocket formed from the gatekeeper residue (Leu210), and also Val147, Leu194 and Leu263. Importantly, the C6-pyridyl-3-yl substituent resides in close proximity to Thr217 of Aurora-A (3.5 Å closest contact), whereas the equivalent residue in Aurora-B/C is a glutamic acid (Fig. 2B). This is one of the three active site sequence differences between Aurora-A and Aurora-B/C. The Leu215 side chain in Aurora-A (arginine in Aurora-B/C) points away from the active site. There is little selectivity to be gained from targeting the side chain of Arg220 (lysine in Aurora-B/C) which is highly mobile and disordered in our co-crystal structure. We exploited this observation in the design of compounds with substantially enhanced selectivity in inhibiting Aurora-A over isoforms -B and -C. It was envisaged that isoform selectivity for Aurora-A could be achieved by the introduction of a C6-phenyl ring bearing an electron rich substituent capable of forming a hydrogen bond with Thr217 in Aurora-A, which would sterically clash with the equivalent residue in Aurora-B/C. On this basis, the sulfonamide derivative **17** (Figs. 2C and 4) was prepared from **14**, by first reacting with 3-(aminomethyl)phenylboronic acid under the conditions described in Scheme 2, and then treating the cross-coupling product with CH_3SO_2Cl in pyridine/CH_2Cl_2.

Compound **17** inhibited recombinant human Aurora-A and Aurora-C with IC_{50} values of 0.060 and 2.35 μM, respectively. In cells, **17** displayed a 70-fold selectivity in inhibiting Aurora-A over Aurora-B, the cellular IC_{50} values determined as 0.15 and 10.54 μM, respectively. In the same cellular assay, we found that the Aurora-A inhibitor MLN8054[18] was 90-fold selective for Aurora-A over Aurora-B. The crystal structure of **17** bound to Aurora-A was determined to a resolution of 2.6 Å, and shows that **17** occupies the ATP-binding site in a mode similar to that observed for **15** (Fig. 2D). The sulfonamide group in **17** is flexible and adopted two conformations in the crystal structure, one of which showed the predicted hydrogen bond interaction with the side chain of Thr217 (Fig. 2E). We propose that the selectivity of **17** for inhibition of Aurora-A is attributable to the interaction of the sulfonamide group with Thr217 in Aurora-A, and the likely clash of this group with the more bulky equivalent glutamic acid in Aurora-B/C. In line with this proposal, the acetamide counterpart of **17** (**18**, Fig. 4) was 46-fold selective in inhibiting Aurora-A in cells. In contrast, the $PhCH_2NH_2$ derivative **19** (Fig. 4) was considerably less selective for Aurora-A in cells compared with **17** (20-fold vs 70-fold). Additional evidence supporting the hypothesis that the observed selectivity is driven by Thr217 came from experiments with T217E mutant Aurora-A. As described earlier, inhibition of Aurora-A autophosphorylation at T288 was used as a biomarker in cellular assays. As shown in Fig. 3, the T217E mutant Aurora-A was resistant to inhibition by **17** and MLN8054, an Aurora-A selective inhibitor. These results are in line with recent studies showing that T217E and T217D mutants of Aurora-A are less sensitive to inhibition by MLN8054.[35,36] It should be noted that Coumar et al. recently reported a pyrazole-based Aurora-A selective inhibitor, and rationalised the selectivity for inhibition of Aurora-A over Aurora-B/C by proposing a similar argument, that is, hydrogen bonding interaction with the backbone NH of Thr217 in Aurora-A and steric clash with the equivalent residue (Glu) in Aurora B/C.[37]

In summary, HTS of our in-house compound collection against recombinant human Aurora-A kinase provided 6,8-disubstituted imidazo[1,2-*a*]pyrazine derivatives as promising hits for a hit-to-lead exploration programme. It was found that the introduction of C3–Cl, C3–Br or C3–Me substituent in **2** improved Aurora-A inhibitory activity by approximately 10-fold. Co-crystallisation of **15** with Aurora-A provided a clear understanding of the interactions for this class of compound with Aurora kinases. Based on this knowledge, we designed compound **17** that showed high

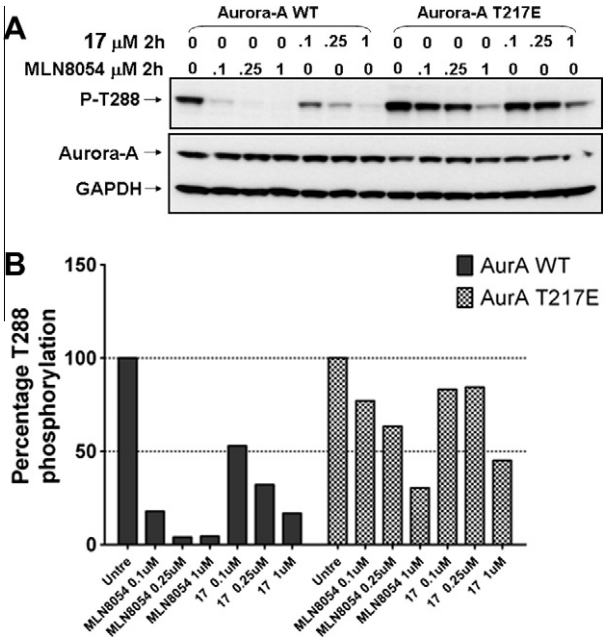
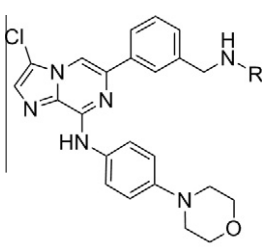


Figure 3. Aurora-A T217E mutant is resistant to inhibition by **17** and MLN8054. (A) Western blot analysis showing inhibition of Aurora-A WT and T217E autophophorylation (P-T288) by **17** and MLN8054. Hela cells transfected with Myc-Aurora-A WT and T217E were treated with indicated concentrations of **17** and MLN8054 for two hours. After protein extraction and separation by SDS–PAGE, autophosphorylation was detected using anti-phosphoThr288 antibodies (AuroraA-pThr288), total Myc-Aurora-A protein was detected using an anti Myc antibody, and anti-GAPDH was used as a loading control. (B) Quantification of the Western blots. P-T288 blots were quantified and normalised to myc-Aurora-A. Results are represented as percentage of untreated.



17. R = SO_2CH_3
18. R = $COCH_3$
19. R = H

Figure 4. Compound **17** and analogues **18** and **19**.

selectivity for Aurora-A in cellular assays, comparable to that of MLN8504. We propose that the observed Aurora isoform selectivity is driven by Thr217 in Aurora-A, the equivalent residue in Aurora-B/C is a glutamic acid. We observed that in the crystal structure of **17** bound to Aurora-A, the sulfonamide group of **17** interacts with Thr217. It was also shown that the T217E mutant Aurora-A is considerably less sensitive to inhibition by compound **17**. Finally, **17** inhibited the HCT116 cell growth with a GI_{50} value of 2.4 μM, and is a useful chemical tool for investigating the role of selective Aurora-A inhibition.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.08.091.

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