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Synthesis and SAR of 1-acetanilide-4-aminopyrazole-substituted quinazolines: Selective inhibitors of Aurora B kinase with potent anti-tumor activity

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Abstract—A new class of 1-acetanilide-4-aminopyrazole-substituted quinazoline Aurora kinase inhibitors has been discovered possessing highly potent cellular activity. Continuous infusion into athymic mice bearing SW620 tumors of the soluble phosphate derivative **2** led to dose-proportional exposure of the des-phosphate compound **8** with a high-unbound fraction. The combination of potent cell activity and high free-drug exposure led to pharmacodynamic changes in the tumor at low doses, indicative of Aurora B-kinase inhibition and a reduction in tumor volume.

The Aurora proteins are a small family of serine/threonine kinases that are expressed during mitosis and have roles in chromosome segregation and cytokinesis. Humans express three Aurora kinase paralogues—Aurora A, B and C, the biology of which has been reviewed extensively.[1–4] With their key role in mitosis and being aberrantly over-expressed in tumor cells, the Aurora kinases have been suggested to be attractive drug targets.[1] Inhibition of either Aurora A or Aurora B leads to very different cellular phenotypes. When Aurora B is inhibited in tumor cells, the cells are forced through a catastrophic mitotic exit leading to polyploid cells that rapidly lose viability. In contrast, selective inhibition of Aurora A-kinase activity results in an accumulation in mitosis and abnormalities in centrosome separation leading to the formation of monopolar spindles.[4]

A number of small molecule inhibitors of the Aurora kinases have demonstrated anti-tumor activity and have subsequently entered clinical evaluation. MK-0457 (VX-680),[5] and PHA-7395358,[6,7] inhibit Aurora A and Aurora B (whilst being more potent inhibitors of Aurora A-kinase activity) and have some activity against other, non-Aurora, kinases.[5–7] Most recently, MLN8054 a selective Aurora A-kinase inhibitor with potent anti-tumor activity has been reported,[8,9] and we have described the discovery and pre-clinical activity of AZD1152, the first Aurora B selective inhibitor to enter clinical evaluation.[10,11]

AZD1152 **1** (Fig. 1) resulted from optimization of a series of 5-acetanilide-3-aminopyrazole (3-pyrazole)-substituted quinazolines which are selective for Aurora B over Aurora A and achieve high levels of solubility by virtue of their ability to be delivered as readily activated phosphate derivatives making them suitable for parenteral administration.[10] AZD1152 possesses robust anti-tumor activity in a range of pre-clinical models and is currently in phase I clinical trials.[11] In this paper we report the synthesis and properties of a series of 1-acetanilide-4-aminopyrazole-substituted quinazolines that

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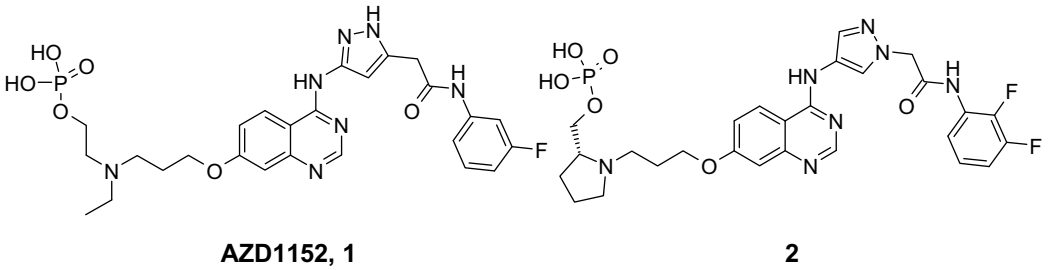


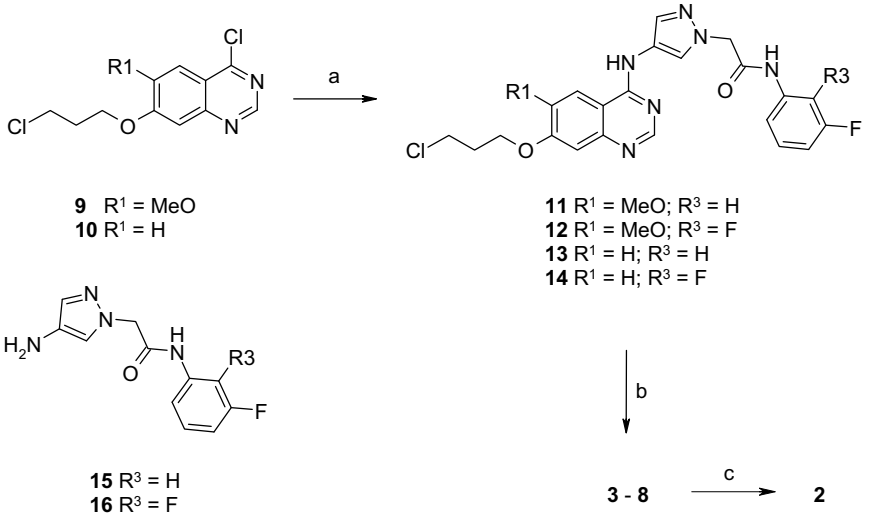
Figure 1. Structures of AZD1152, **1** and 4-pyrazole compound, **2**.

led to the discovery of compound **2** (Fig. 1) and which complements the other series we have described previously.[10–15]

Compounds **2**–**8** were prepared as shown in Scheme 1.[16] The amino pyrazoles **15** and **16**,[16,17] were reacted with the 4-chloroquinazoline intermediates **9** and **10**,[10] to give the chloropropoxy pyrazole acetanilide intermediates **11**–**14**. The drug compounds **3**–**8** were then prepared by displacing the chloro-leaving group in **11**–**14** with the appropriate amine under standard conditions. The phosphate derivative **2** was prepared using an analogous procedure described previously.[10]

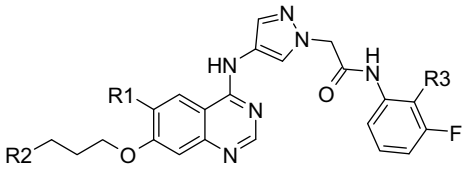
The SAR in the 1-acetanilide-4-aminopyrazole (4-pyrazole) series followed the same broad trends we have reported for the related 5-acetanilide-2-aminothiazole (thiazole),[15] and 3-pyrazole series.[10] A representative set of compounds is shown in Table 1.[16] In summary, the 4-pyrazole compounds are highly potent Aurora B-kinase inhibitors but show less activity against Aurora A. Fluoro groups are particularly favorable especially in the ortho- and meta-positions and a number of basic side chains attached to the C-7 position of the quinazoline ring delivered potent cellular activities. Selectivity for Aurora B over Aurora A is enhanced in compounds where the methoxy group at the C-6 position on the quinazoline ring is replaced with a proton. In matched pair analogues, compounds lacking the C-6 methoxy substituent achieve significantly increased inhibition of histone-H3 phosphorylation in human SW620 colorectal adenocarcinoma tumor cells (compare compounds **5** vs **7** and **3** vs **8** in Table 1). Histone-H3 is a cellular substrate of Aurora B and a marker of Aurora B-kinase inhibition in vivo. The cell activity for the 4-pyrazole compounds is particularly noteworthy and even greater than the corresponding 3-pyrazole analogues.[10] In the 4-pyrazole series multiple compounds were discovered with a cellular $EC_{50} < 10$ nM and some, such as **8**, with a cellular $EC_{50} < 1$ nM.

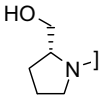
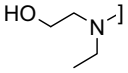
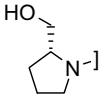
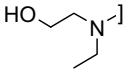
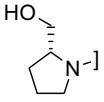
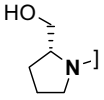
In contrast to the 3-pyrazole series, compounds from the 4-pyrazole series potently inhibit a small number of other kinases while retaining generally high specificity for Aurora B. The kinase activity profile for compound **8** in a panel of 62 kinases is shown in Figure 2. Four kinases (PDGFRα, β, CSF-1R and c-KIT) are inhibited with IC_{50} values within 100-fold of the Aurora B-INCENP value (IC_{50} values = 0.069, 0.006, 0.036 and 0.009 μM, respectively). Despite the existence of other non-Aurora kinase activities, SW620 cells treated with compound **8** show reduced histone-H3 phosphorylation and undergo endoreduplication, a phenotype consistent with the inhibition of Aurora B kinase.[4,12]



Scheme 1. Reagents and conditions: (a) **15** or **16**, 4.0 N HCl (dioxane), DMA, 50–90 °C, 69–95% yield; (b) Amine, KI, DMA, 90 °C, 58–91% yield; (c) di-*tert*-butyldiethylphosphoramidite, tetrazole, DMF, rt; H_2O_2, −10 °C → rt; HCl, dioxane, rt; 68% yield over 3 steps.

Table 1. Summary of SAR in the 4-pyrazole series



Compound	R^1	R^2	R^3	Aurora A K_i[a,c] (μM)	Aurora B-INCENP K_i[a] (μM)	Cell EC_{50}[a,b] (μM)
3	MeO		F	0.34	<0.001	0.023
4	MeO		F	0.54	<0.001	0.010
5	MeO		H	1.3	0.001	0.089
6	MeO		H	1.1	0.001	0.032
7	H		H	3.0	<0.001	0.007
8	H		F		<0.001	<0.001

[a] Average of at least two independent dose–response curves. Variation was generally <25%.
[b] Inhibition of histone-H3 phosphorylation following 24-h incubation in SW620 tumor cells. Assays were carried out as described in Ref. 10.
[c] K_i value for compound **8** was not measured.

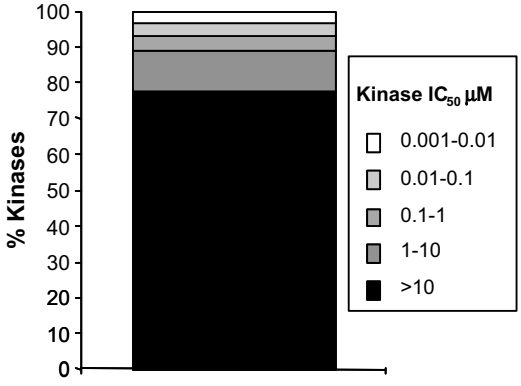


Figure 2. Activity profile for compound **8** in a panel of 62 serine/threonine and tyrosine kinases.

Compounds from the 4-pyrazole, like the 3-pyrazole series,[10] possess attractive physicochemical, pharmacokinetic and safety-pharmacology properties; selected data for compounds **8** and **2** are summarized in Table 2. In our previous reports we have shown a role for the charged side chain, in compounds such as **8**, in reducing Log *D* and concomitantly enhancing potency.[10,13,15] In addition to the charged side chain, the heterocycle linking the hinge (quinazoline) to the lipophilic pocket (benzamide)-binding group, has a further effect on physicochemical-related properties. Both pyrazole-based series have significantly reduced protein binding compared to the corresponding thiazole analogues (Fig. 3). Lipophilicity is likely to play a key role in this observed difference with the potential for an additional structural component.

Table 2. Physicochemical, pharmacokinetic and safety-pharmacology properties of compounds **8** and **2**

Compound	Log $D_{7.4}$	pK_a	Solubility[a] (mg/mL)	% Bound[b]	Rat Cl[c] (mL/min/kg)	3A4 inhibition IC_{50}[d] (μM)	hErg IC_{50}[e] (μM)
8	2.1	8.9, 5.4		91	40	>10	>100
2			10			6.1	>100

[a] Tris buffer, pH 9.
[b] Rat serum albumin.
[c] Compounds were dosed to male Han Wistar rats at 5 mg/kg formulated in a mixture of 10% DMSO in water.
[d] The activity of human CYP3A4 determined by inhibiting the biotransformation of 7-benzyloxy-4-(trifluoromethyl)-coumarin to the fluorescent metabolite 7-hydroxy-4-trifluoromethyl-coumarin.
[e] Activity against the human ether-a-go-go-related gene (hERG)-encoded potassium channel was determined using automated whole-cell electrophysiology.

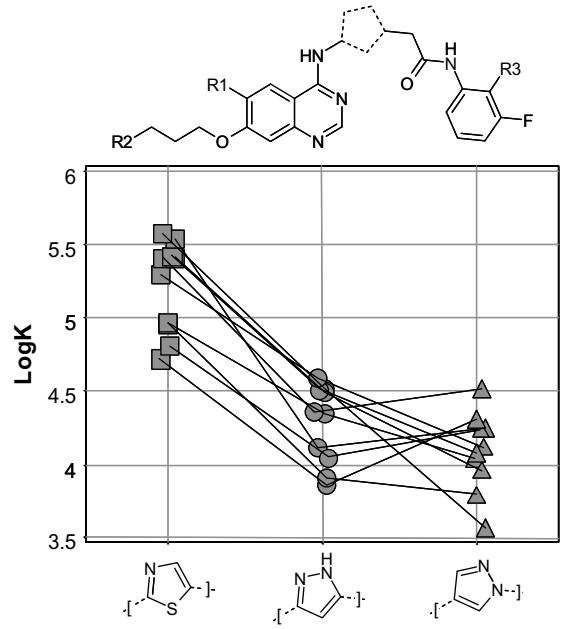


Figure 3. Log K values measured in rat plasma for matched-pair analogues in the thiazole (■),[15] 3-pyrazole (●),[10] and 4-pyrazole (▲) series. Each marker represents an individual compound matching the substructure shown. Lines connect exact (R^1, R^2, R^3) matches. Average Log K values for thiazole, 3-pyrazole and 4-pyrazole compound sets in the study = 5.2, 4.3 and 4.1, respectively.

Following subcutaneous infusion of the phosphate **2**, the plasma concentration of the phosphate **2** and the parent drug **8** increased in parallel with increasing dose (Fig. 4). To determine the rate and extent of conversion, an iv-bolus of phosphate **2** was administered in rats. The peak mean plasma concentration (C_{max}) of both **2** and the parent **8** occurred at 2 min postdose (t_{max}), indicating that **2** was rapidly converted to the parent drug **8** (Fig. 5). When compound **2** was dosed as an iv-bolus, it provided 98% of the AUC of the parent **8** compared with direct dosing of **8**. These data demonstrate that conversion of the phosphate prodrug to the parent drug is both rapid and complete following parenteral administration. Interestingly, both the parent compound **8** and

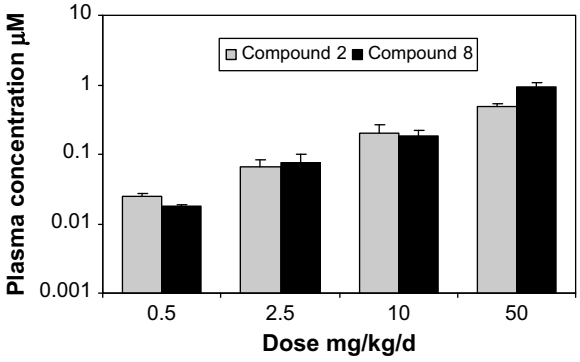


Figure 4. Plasma concentration of compounds **2** and **8** following dosing of compound **2** in male Swiss nude (*nu/nu* genotype) mice. Compound **2** was administered as a constant infusion for 48 h from a subcutaneous osmotic mini-pump. Columns, plasma concentration measured immediately at end of infusion period µM; bars, SEM.

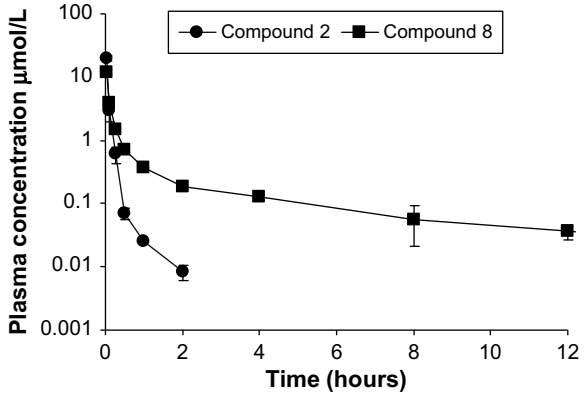


Figure 5. Group mean plasma concentration (±SEM) of compound **2** and compound **8** in male Wistar Hannover rats following single intravenous bolus dosing of compound **2** at 5 mg/kg.

the phosphate **2** have potent anti-proliferative effects in SW620 cells (IC_{50} < 0.001 µM).[18] However, compounds bearing phosphate groups are considered to be impermeable due to the highly charged nature of the phosphate moiety at physiological pH.[19] The cellular activity observed for the phosphate **2** can be rationalized by conversion to the parent compound **8** during the 48-h incubation period of the assay.[20]

The combination of high cellular potency, a high-unbound fraction and good exposure for compound **8** following dosing of the phosphate prodrug **2** led to in-vivo activity at low doses. Inhibition of histone-H3 phosphorylation at doses of **2** from 0.5 mg/kg/d was observed reaching a maximum of 75–85% inhibition from doses of 2.5 mg/kg/d onwards (Fig. 6). At all of the doses tested, inhibition of histone-H3 phosphorylation correlated with an accumulation of cells with 4 N DNA content compared with vehicle-treated controls. These

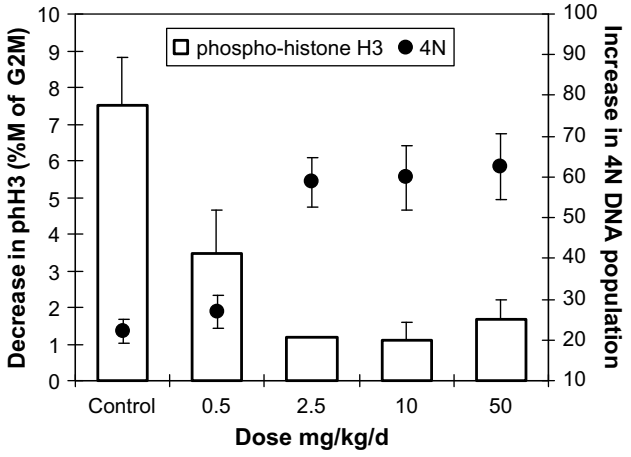


Figure 6. Pharmacodynamic activity of compound **2** in SW620 tumor xenografts established in male nude mice. Compound **2** at the doses indicated or vehicle were administered as a constant infusion for 48 h using a subcutaneous osmotic mini-pump. Figure shows flow-cytometric analysis for phospho-histone-H3 (PhH3) (Columns, percentage of PhH3 positive cells gated in G2/M phase of cell cycle; bars, SD) and 4 N DNA content (Points, percentage 4 N DNA cells; bars, SD).[11]

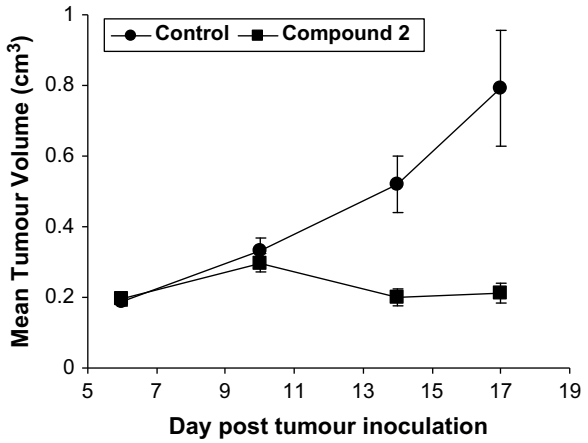


Figure 7. In vivo tumor-growth inhibition activity of compound **2** in SW620 tumor xenografts. Male nude mice ($n = 9$–10 per group) were dosed with either vehicle or a single cycle of compound **2** (50 mg/kg/day) as a constant infusion for 24 h using a subcutaneous osmotic mini-pump from day 6 to day 7. Points, mean tumor volume cm^3; bars, SEM.[11]

results are consistent with inhibition of Aurora B-kinase activity in the tumors and recapitulate the in-vitro phenotypic observations.[10,11]

The robust pharmacodynamic activity of compound **2** led to significant inhibition of SW620 tumor growth in nude mice. At the higher doses, mechanism-related and reversible myelosuppression was found to be the dose-limiting toxicity. However, anti-tumor activity was seen for compound **2** using a tolerated schedule of 50 mg/kg/d infusing for 24 h which gave a reduction in tumor size of 89% at the end of the study compared to tumors in vehicle-treated control animals (Fig. 7). A reduction in tumor size was also seen at doses as low as 2.5 mg/kg/d dosed for 48 h (data not shown).

In summary, we have discovered a new series of aminopyrazole-substituted quinazoline Aurora kinase inhibitors. Optimization led to the discovery of compound **2**, which in pre-clinical in-vivo models shows activity at lower doses compared with other series we have previously reported.

Acknowledgments

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18. Compounds were incubated with SW620 tumor cells in the presence of 5% foetal-calf serum for 48-h. The antiprolif-

erative effect of compounds was measured by inhibition of DNA synthesis using a 2-h BrdU uptake assay.
19. Pharmaceutical interest in phosphate esters (especially in the antiviral area) has necessitated the development of prodrugs of the phosphate moiety to deliver such compounds into target cells e.g. see: (a) Schultz, C. *Bioorg. Med. Chem.* **2003**, *11*, 885; (b) Jones, R. J.; Bischofberger, N. *Antiviral Res.* **1995**, *27*, 1.
20. The phosphonate analogue of **1** retains activity vs Aurora B kinase but does not have significant activity in cellular assays ($IC_{50} > 1\ \mu M$) confirming that highly charged compounds have low cell permeability.