Discovery, Synthesis, and *in Vivo* Activity of a New Class of Pyrazoloquinazolines as Selective Inhibitors of Aurora B Kinase

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Received November 17, 2006

The Aurora kinases have been the subject of considerable interest as targets for the development of new anticancer agents. While evidence suggests inhibition of Aurora B kinase gives rise to the more pronounced antiproliferative phenotype, the most clinically advanced agents reported to date typically inhibit both Aurora A and B. We have discovered a series of pyrazoloquinazolines, some of which show greater than 1000-fold selectivity for Aurora B over Aurora A kinase activity, in recombinant enzyme assays. These compounds have been designed for parenteral administration and achieve high levels of solubility by virtue of their ability to be delivered as readily activated phosphate derivatives. The prodrugs are comprehensively converted to the des-phosphate form *in vivo*, and the active species have advantageous pharmacokinetic properties and safety pharmacology profiles. The compounds display striking *in vivo* activity, and compound **5** (AZD1152) has been selected for clinical evaluation and is currently in phase 1 clinical trials.

Introduction

The Aurora proteins are a small family of serine/threonine kinases that are expressed during mitosis and have been suggested to be attractive drug targets.[1] They have roles in chromosome segregation and cytokinesis and ensure that a complete copy of the duplicated genome is precisely partitioned into the two daughter cells. Humans express three Aurora kinase paralogues, Aurora A, B, and C, the biology of which has been reviewed extensively.[1,2] With their key role in mitosis and being aberrantly overexpressed in tumor cells, there has been considerable interest in developing specific Aurora kinase inhibitors and in understanding the therapeutic value of selectively inhibiting Aurora A and B.

While there are now considerable data supporting a link between Aurora A and B kinase expression and cancer (the role of Aurora C is currently poorly understood[3]), it is still unclear whether inhibition of Aurora A and/or Aurora B, could be advantageous in terms of providing therapeutic benefit in oncology.[4] Studies using both pharmacological and genetic disruption of Aurora kinases in cells have shown that the mitotic defects described following exposure of cells to Aurora kinase inhibitors seem largely due to inhibition of Aurora B.[1,5−8] 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, inhibition of Aurora A kinase activity causes a mitotic delay and abnormalities in centrosome separation leading to the formation of a monopolar spindle.[8]

A number of small molecule inhibitors of Aurora kinase activity, from structurally diverse chemical series, have already been reported and/or reviewed elsewhere.[4,9−18] VX-680[9] was the first inhibitor reported to show potent antitumor activity *in vivo*; similar results for PHA-680632[16] and PHA-739358[17] have also been reported recently. All of these drugs inhibit Aurora A and Aurora B (while being more potent inhibitors of Aurora A kinase activity) and have some activity against other, non-Aurora, kinases.[9,16,17,19] To date, no reports of Aurora B-selective inhibitors have appeared although the first disclosure of an Aurora A-selective inhibitor has been reported.[20]

In this paper we describe the discovery of a novel series of Aurora kinase inhibitors that are specific for Aurora kinases over other kinases and which show high selectivity for Aurora B over Aurora A. Compounds from this series have attractive physicochemical and pharmacokinetic properties and have been demonstrated to possess potent antitumor activity in preclinical models.

Chemistry. Compounds **5**−**39** were prepared as shown in Scheme 1. To explore the SAR of the lipophilic pocket binding acetanilide group, we first prepared the intermediate **43** by displacing the chloro leaving group in **41** with 4-(hydroxymethyl)piperidine. The acid group in **43** was then further reacted with a range of substituted anilines, using EDC[a] and 2-hydroxypyridine 1-oxide to give the acetanilides **6**, **7**, **9**, and **12**−**17**. To explore the SAR in the quinazoline C-7 side chain, we first made the chloropropoxy acetanilide intermediates **44**−**48**, from the acids **41** and **42**. Compounds **44**−**47** were prepared using either EDC and 2-hydroxypyridine 1-oxide or $POCl_3$ and pyridine, in moderate to excellent yields. Compound **48** was prepared by reacting **42** with pentafluorophenyl trifluoroacetate to first give the pentafluorophenyl ester which was subsequently reacted with 3-fluoroaniline to give **48** in excellent yield without

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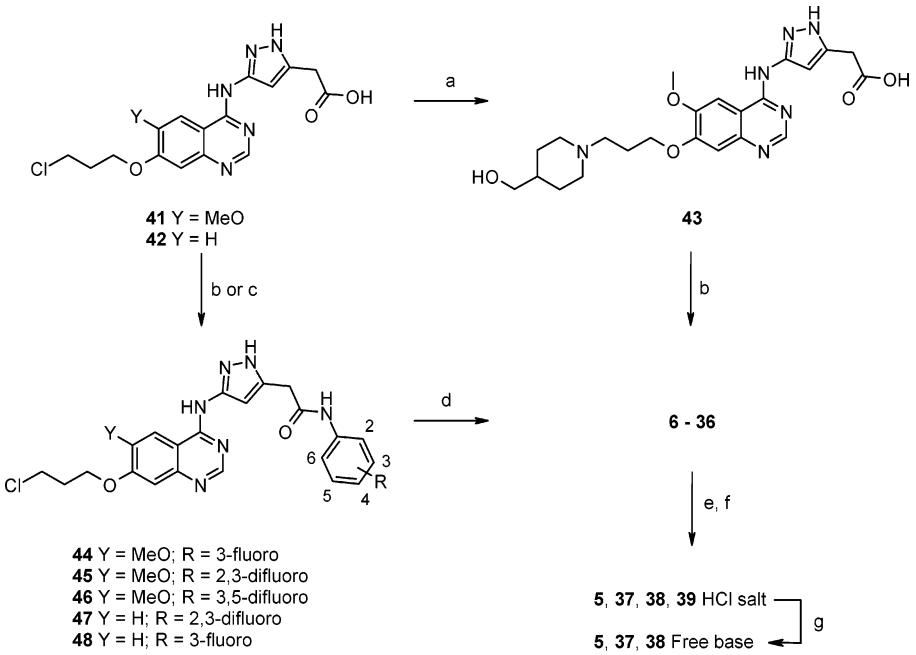
[a] Abbreviations: DMA, *N*,*N*-dimethylacetamide; DMF, *N*,*N*-dimethylformamide; DMSO, dimethyl sulfoxide; EDC, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride; NMP, 1-methyl-2-pyrrolidinone; TFA, trifluoroacetic acid.

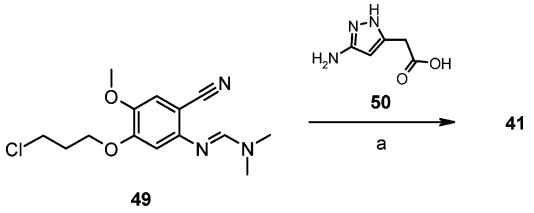
10.1021/jm061335f CCC: $37.00
Published on Web 03/21/2007

Scheme 1[a]



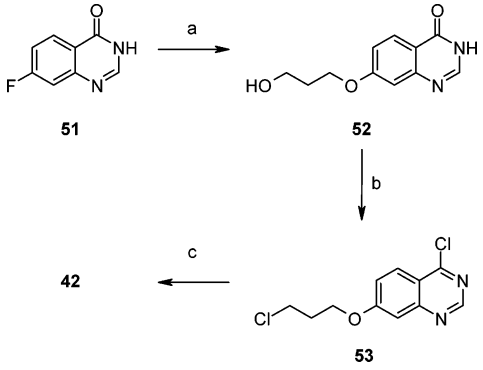
[a] Reagents: (a) 4-(hydroxymethyl)piperidine, DMA, 100 °C; (b) $ArNH_2$, DMA, EDC, 2-hydroxypyridine 1-oxide, 50 °C; (c) $ArNH_2$, pyridine, $POCl_3$, 0 °C → r.t. or $ArNH_2$, pentafluorophenyl trifluoroacetate, pyridine, DMF, 0 °C → r.t.; (d) amine, DMA, 90 °C or amine, NMP, KI, 60 °C or amine, DMA, KI, 50 °C; (e) di-*tert*-butyldiethylphosphoramidite, tetrazole, DMF, r.t.; H_2O_2, −10 °C → r.t.; (f) HCl, dioxane, r.t.; (g) cyclohexene oxide, MeOH.

Scheme 2[a]



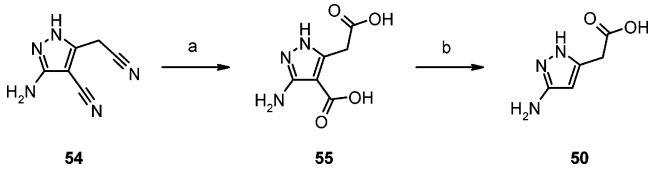
[a] Reagents: (a) AcOH, **50**, reflux.

Scheme 3[a]



[a] Reagents: (a) NaH, 1,3-propanediol, DMF, 60 → 110 °C; (b) $SOCl_2$, DMF, 85 °C; (c) **50**, DMF, HCl (dioxane), 90 °C.

Scheme 4[a]



[a] Reagents: (a) 12 M NaOH, 100 °C; (b) H_2O, reflux.

the need for chromatography and on a multigram scale. Finally, the drug compounds **8**, **10**, **11**, and **18**−**36** were prepared by displacing the chloro leaving groups in **44**−**48** with the appropriate amine, using a range of standard conditions. The phosphate prodrugs **5**, **37**, **38**, and **39** were prepared as described previously.[15] Thus the free hydroxyl group in compounds **18**, **29**, **33**, and **34** was first reacted with di-*tert*-butyldiethylphosphoramidite and tetrazole in DMA to give the phosphite esters. These were then oxidized in the same pot with an aqueous solution of hydrogen peroxide to give the corresponding *tert*-butyl phosphate esters. The *tert*-butyl groups were then cleaved with hydrogen chloride in dioxane to afford the phosphate prodrugs **5**, **37**, **38**, and **39** as dihydrochloride salts. The hydrochloride salts could be optionally desalted with cyclohexene oxide in methanol to generate the free base.

The intermediate compounds **41** and **42** were prepared as shown in Scheme 2 and Scheme 3, respectively. Compound **41** was prepared from the *N*,*N*-dimethylimidoformamide compound **49**, which we have described previously,[15] following Dimroth cyclization and rearrangement with the aminopyrazole **50** in refluxing acetic acid. The quinazoline C-6-unsubstituted, pyrazole acetic acid intermediate **42** was prepared by an alternative sequence as shown in Scheme 3. The mono anion of 1,3-propanediol was reacted with 7-fluoroquinazolinone **51**,[21] to give the alcohol **52** in high yield. The alcohol functionality and quinazolinone groups in **52** were chlorinated in a single step using hot thionyl chloride and DMF as catalyst to give the dichloro compound **53**. Finally, **53** was reacted with the aminopyrazole **50** under acidic conditions to give **42** in good yield and high purity without the need for chromatography.

The aminopyrazole starting material **50** can be prepared in multigram quantities in two steps from commercially available 5-amino-3-(cyanomethyl)-1*H*-pyrazole-4-carbonitrile **54** (Scheme 4). Hydrolysis of the nitrile groups in **54** was achieved with concentrated sodium hydroxide at 100 °C followed by neutralization with hydrochloric acid to give the diacid **55**. Finally, **55** underwent facile decarboxylation in refluxing water to give **50** in high yield over the two stages.

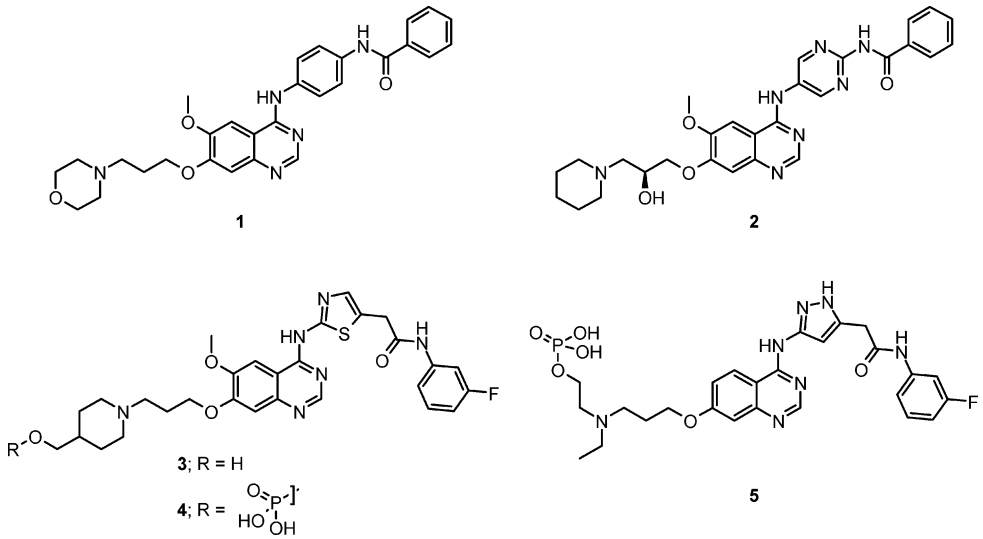


Figure 1. Compounds from the AstraZeneca Aurora kinase inhibitor series.

Discussion

In previous publications we have described a number of quinazoline-based Aurora kinase inhibitor series.[5,10,14,15] The *p*-benzamidoanilinoquinazoline series, e.g., ZM447439 (**1**) (Figure 1), was identified following a high throughput screening campaign. Compound **1** was shown to inhibit Aurora A and Aurora B equipotently but in a panel of other serine/threonine and tyrosine kinases, showed high specificity for Aurora kinases.[5] Compound **1** has sub-micromolar activity in cellular proliferation assays but relatively poor drug-like properties.[10]

Investigation of the SAR around the anilino ring linking the key hinge (quinazoline) and lipophilic pocket (benzamide) binding groups in **1** showed that this ring could be replaced with a number of heterocyclic systems to give improvements in both potency and physicochemical properties.[10] Furthermore, it was found from both the testing of small libraries and from in-house crystallographic studies that Aurora A is able to accommodate highly polar functional groups within the side chain at the quinazoline C-7 position. Compounds with elaborated C-7 side chains show good cellular activity and benefit from much better solubility and free drug levels.[10] The structure−activity relationships of the pyridino- and pyrimidinoquinazoline compounds have been described recently and includes one of the first crystal structures of an Aurora kinase with a bound small molecule inhibitor, the pyrimidinoquinazoline **2**.[10,14] The X-ray structural determination of compound **2** in Aurora A was helpful in rationalizing the observed high potency and high specificity for the Aurora kinases.[14]

More recently we have described a series of thiazoleacetanilide inhibitors of Aurora kinase that show further improvements in cellular activity and which included compound **3**.[10,15] When compound **3** was administered as its dihydrogen phosphate derivative **4**, a significant inhibition of histone H3 phosphorylation in human SW620 colorectal adenocarcinoma tumors could be demonstrated.[10,15] Histone H3 is a cellular substrate of Aurora B and is therefore a useful marker of Aurora B kinase inhibition *in vivo*.[5]

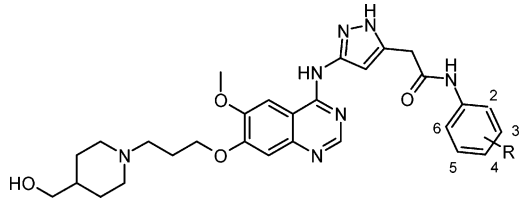
While the identification of a compound with *in vivo* activity against a relevant biomarker represented significant progress, this activity was somewhat variable, and antitumor studies with this compound (SW620 tumors grown in nude mice) were ultimately inconclusive. However, in parallel to the thiazole series we were also evaluating alternative chemical starting points. In this paper we describe some of our further efforts to identify Aurora kinase inhibitors suitable for clinical evaluation. In particular, we report the synthesis, structure−activity relationships (SAR), and much improved *in vivo* activity of a novel series of 5-acetanilide-substituted 3-aminopyrazoles. A compound from this series, compound **5** (AZD1152), has been selected for clinical evaluation.[22]

The phosphate derivatives (e.g., compound **4**) have very high aqueous solubility (>10 mg/mL) in simple pH-adjusted saline without the need for recourse to complex with potentially highly toxic vehicles. This high level of solubility allowed us to explore the effects of both short- (bolus) and longer-term exposure of our compounds in preclinical models and hence to understand the complex balance between concentration and duration of exposure on efficacy and tolerability.[23]

While a number of highly potent compounds were made in this pyrazole series which lacked a suitable hydroxyl group for attaching a solubilizing phosphate group,[24] all the compounds disclosed in this paper were designed for parenteral administration as the corresponding dihydrogen phosphate prodrugs.[24−26] For clarity of discussion, data are presented for a limited, representative set of compounds (further compounds have been described in patent applications).[24−26] It should be borne in mind that the structure−activity relationships described in the paper have come from analysis of the full set of compounds. Thus, where trends are exemplified by a single pair of compounds in the tables, the conclusions presented are typically supported by data from additional compounds. For clarity we have chosen to represent the pyrazole ring in this paper in a single tautomeric form. It is likely that both tautomers can exist but the relative contribution to binding of each form remains undetermined.

It was found that the pyrazole series are highly potent Aurora B kinase inhibitors but generally show less activity against Aurora A, in assays using recombinant enzymes (Table 1). Knowledge of the SAR from earlier series suggested that small and lipophilic groups were particularly favored in the terminal lipophilic binding pocket.[10,15] It was found that the SAR in the pyrazole series followed these same broad trends, so a small focused library of compounds was made where a small number of preferred substitution patterns were emphasized (Table 1). Fluoro groups were found to be particularly favorable especially in the ortho- and meta-positions (e.g., compounds **7**, **8**, **10**, and **11** compared with compound **9**; Table 1). The most favored aniline substituents, in terms of Aurora B kinase activity and inhibition of histone-H3 phosphorylation in SW620 cells are

Table 1. Acetanilide SAR in the 3-Aminopyrazole Series



No.	R	Aurora A K_i, μM[a]	Aurora B-INCENP K_i, μM[a]	Cell IC_{50}, μM[a,b]
6	H	1.6	0.018	1.2
7	2-F	1.4	0.014	0.47
8	3-F	0.45	0.002	0.68
9	4-F	2.0	0.030	2.6
10	2,3-*di*-F	0.41	0.002	0.54
11	3,5-*di*-F	0.23	0.001	0.37
12	3-Cl	0.45	0.008	1.2
13	3-CN	2.6	0.083	>10
14	3-NO_2	0.89	0.039	>10
15	3-OH	4.0	0.14	>10
16	3-OMe	1.9	0.009	5.5
17	3-CF_3	0.51	0.013	1.8

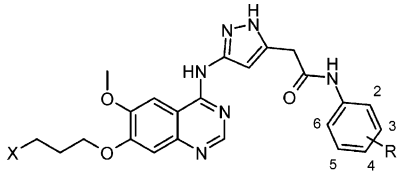
[a] Average of at least two independent dose−response curves. Variation was generally <25%. [b] Inhibition of histone-H3 phosphorylation following 24 h incubation in SW620 tumor cells.

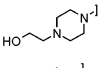
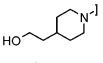
3-fluoro, 2,3-difluoro, and 3,5-difluoro (compounds **8**, **10**, and **11**, respectively). The 2,3-difluoro and 3-fluoro acetanilide groups generally give rise to better physicochemical properties. Larger and more polar functional groups are also tolerated but are less preferred, especially in terms of cellular potency.[27] These data suggest the binding conformations of the thiazole and pyrazole series are likely to be comparable;[15] however, the presence of a further hydrogen bond donor in the pyrazole ring (compared to the thiazole ring) may allow additional binding to the protein backbone although this is difficult to model given that the pyrazole hydrogen could be on either of the two ring nitrogens.

Despite the similarities in the putative binding conformation between the thiazole and pyrazole series, there exists a significant difference in cellular inhibition of phosphorylation of histone H3 (at serine 10) between compound **3** (IC_{50} = 0.005 μM)[15] and the direct 3-aminopyrazole analogue compound **8** (Table 1: IC_{50} = 0.68 μM) in SW620 tumor cells. Both the thiazole and pyrazole series are clearly highly potent inhibitors of Aurora B kinase enzyme activity. The cellular difference between compounds **3** and **8** may be attributable to a combination of subtle differences in binding conformation together with differences in the biophysical characteristics of the two compounds. The pyrazole compounds are generally much less lipophilic than the corresponding thiazole analogues (e.g., measured log $D_{7.4}$ = 3.2 vs 2.1 for compounds **3** and **8** respectively) resulting in an improvement in general physicochemical properties and a decrease in protein binding (98% vs 94% bound for compounds **3** and **8**, respectively, in rat plasma). The improved physical properties, together with reasonable enzyme and cell activity, made the pyrazole series an attractive proposition for further medicinal chemistry aimed at optimizing cellular potency while at the same time maintaining drug-like properties.

Knowledge from previous work suggested that optimization of the quinazoline C-7 side chain might result in further enhancement of cellular potency.[10,15] The presence of the amino functional group increases potency while its basicity helps to reduce the compound's binding to plasma albumin.[10] With a strategy requiring synthesis of *in vivo* hydrolyzable phosphate esters (to obtain high solubility for parenteral administration), the C-7 side chain was also required to contain a pendent hydroxyl functional group.[28] This hydroxyl group plays a second critical role in the drug as, depending on its proximity to the basic nitrogen, it exerts a powerful role in moderating the basicity of the latter group.[29]

Table 2. Optimization of the Quinazoline C-7 Side Chain



No.	R	X	LogD$_{7.4}$[a]	Aurora A Ki, μM[b]	Aurora B-INCENP Ki, μM[b]	Cell IC_{50}, μM[b,c]
18	2,3-*di*-F		2.0	0.094	0.001	0.042
19	2,3-*di*-F			0.039	<0.001	0.045
20	2,3-*di*-F			0.055	<0.001	0.071
21	2,3-*di*-F			0.22	<0.001	0.078
22	2,3-*di*-F			0.28	<0.001	0.13
23	3-F			0.11	0.001	0.33
24	3-F			0.69	0.001	0.63
25	3-F			0.065	<0.001	0.12
26	3-F		2.1	0.19	0.001	0.27
27	3-F			0.22	0.002	0.29
28	2,3-*di*-F		1.9	0.36	<0.001	0.045
29	2,3-*di*-F		2.3	0.087	<0.001	0.018
30	2,3-*di*-F		3.6	0.018	<0.001	<0.001

[a] Measured using shake-flask methodology with a buffer:octanol volume ratio of 100:1. The concentration of compound in the aqueous phase before and after partitioning with octanol was determined by generic HPLC-UV analysis. [b] Average of at least two independent dose−response curves. Variation was generally <25%. [c] Inhibition of histone-H3 phosphorylation following 24 h incubation in SW620 tumor cells.

In the related aniline, pyrimidine, and thiazole series we have demonstrated that the length of the aliphatic spacer, between the oxygen atom on the C-7 position of the quinazoline ring and the basic nitrogen atom within the side chain, governs the ability of the polar group to solvate.[10,15] Thus, if the spacer is too short or too sterically demanding, the polar group is brought too close to the protein and the resultant clash causes a reduction in potency. Two-carbon spacers are well-tolerated but the presence of a second β-oxygen atom may reduce the basicity of the nitrogen in the side chain too much so that, in general, compounds with three carbon spacers were the most studied. Table 2 shows the SAR of selected compounds substituted with a range of basic side chains utilizing a three-carbon spacer.

Both cyclic and noncyclic amine side chains are tolerated by the Aurora kinases, giving rise to excellent levels of cellular potency, in most cases, without a significant increase in

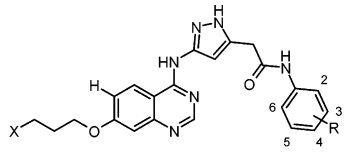
lipophilicity (Table 2). Again, these compounds are highly potent Aurora B kinase inhibitors but show less activity against Aurora A kinase. A range of five- and six-membered cyclic amines (pyrrolidine, piperidine, and piperazine) were prepared (e.g., compounds **18**−**23** in Table 2). The prolinol side chains (compounds **18** and **19**) were found to have the greatest cellular activity with little noticeable difference in terms of potency, between the two enantiomers. Six-membered cyclic amines (e.g., compounds **20**, **21**, and **22**) were found to be marginally less active in cells. In the acyclic series, N-substituted ethanolamines were found to be optimal with a clear relationship between the lipophilicity of the N-substituent and cellular potency (compounds **24**−**30** in Table 2) with the *N*-isobutyl-substituted compound **30** having an IC_{50} in SW620 cells of less than 1 nM. However, we found that as lipophilicity and cellular potency increased, protein binding increased concomitantly. In addition, compounds with high lipophilicity were found to be more likely to inhibit the major P450 isoforms, especially against 3A4 (e.g., compare compound **30**: log $D_{7.4} = 3.6$; 3A4 $IC_{50} = 0.4$ μM vs compound **28**: log $D_{7.4} = 1.9$; 3A4 $IC_{50} > 10$ μM). Within this set of compounds the 2,3-difluorophenyl group gave greater cellular potency compared with the 3-fluorophenyl group. The 2,3-difluorophenyl group also resulted in lower protein binding (compare compounds **18** vs **23** and **28** vs **26**: 85% vs 95% bound and 83% vs 95% bound in rat plasma, respectively). By appropriate combination of the quinazoline C-7 side chain and anilide groups, as described, it is possible to balance attractive physicochemical-based properties (high free drug levels and no P450 inhibition) with high potency in cell assays.

Important roles for the quinazoline C-7 side chain and the lipophilic pocket binding group had been established in the earlier series, and these roles and the associated SAR also broadly applied to the new pyrazole series, albeit with some detailed differences. In contrast, the role of the substituent at the C-6 position on the quinazoline ring was less well understood. It was hypothesized that this group was not involved in direct binding to the protein (although it did have the potential to influence the hydrogen bond acceptor properties of the quinazoline ring in its key interaction with the hinge) and that its removal would allow a reduction in molecular weight and remove any risk of reactive metabolites being formed resulting from oxidative metabolism of the methyl group. Surprisingly, it was found that replacing the C-6 methoxy group with a proton generally reduced potency versus Aurora A kinase but maintained very high potency versus Aurora B kinase; improved potency was also observed in the SW620 cell assay (compounds **31** to **36** in Table 3).

Compounds such as **34** and **36** show 1000-fold greater potency for Aurora B than for Aurora A, in the recombinant enzyme assays. The cellular phenotype observed for these Aurora B-selective compounds is identical to that described previously for compound **1** and other Aurora kinase inhibitors.[5,6,9] The Aurora B-selective inhibitors, such as compound **34**, inhibit phosphorylation of histone H3 on serine 10 and result in a failure of cell division and polyploidy without affecting the core cell cycle timing through mitosis.[30] The Aurora-B selective compounds also retain the high specificity for Aurora kinases demonstrated in our earlier series. For example, in a panel of other serine/threonine and tyrosine kinases, compound **34** showed very little other kinase activity (Table 4) with the exception of Aurora C-INCENP.

A range of compounds, from this new series, spanning the chemical diversity shown in Tables 1−3, were considered for *in vivo* evaluation based on their cellular activity, free drug exposure, and absence of key safety pharmacology liabilities (such as inhibition of the hERG ion-channel and the major cytochrome P450 isoforms). Discriminating data for four representative compounds are shown in Table 5. Overall, the compounds in the pyrazole series have moderate lipophilicity and excellent free drug levels and show little or no significant inhibition of any of the five major cytochrome P450 isoforms. Despite the presence of a basic side chain (pK_a of side chain ~9 for compounds in Tables 2 and 3), the compounds from this class do not significantly inhibit the hERG ion-channel at concentrations up to 30 μM when measured in a whole cell

Table 3. Quinazoline C-6 H SAR



No.	R	X	Aurora A Ki, μM[a]	Aurora B-INCENP Ki, μM[a]	SW620 IC_{50}, μM[a,b]
31	2,3-*di*-F		0.16	<0.001	0.007
32	2,3-*di*-F		0.35	<0.001	0.002
33	2,3-*di*-F		0.95	<0.001	0.005
34	3-F		1.4	<0.001	0.017
35	3-F		0.98	<0.001	0.007
36	3-F		1.4	<0.001	0.024

[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.

Table 4. Selectivity Profile (IC_{50}, μM) of Compound **34** in a Panel of Kinases

kinase	IC_{50}, μM[a]	kinase	IC_{50}, μM[a]
Aurora A	1.4	KDR	1.8
Aurora B-INCENP	<0.001	PHK	1.8
Aurora C-INCENP	0.017	ZAP70	8.2
LCK	0.17	others[b]	>10

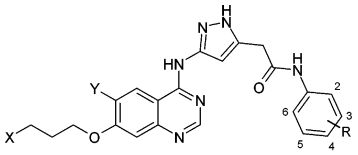
[a] Non-Aurora kinase assays were carried out as described in ref 15. [b] JAK3, vABL, CSK, FAK, SRC, IGFR, EGFR, FGFR, P38A, PAK1, CDK2, JNK1A, PKA, MEK, CHK1, PLK1.

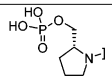
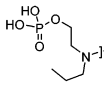
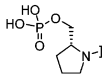
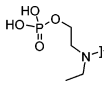
Table 5. Physicochemical and Pharmacokinetic Properties of Selected Compounds from the Pyrazoloquinazoline Series

No.	log $D_{7.4}$[a]	% bound[b]	rat Cl, mL/min/kg[c]	3A4 inhibition IC_{50}, μM[d]	hERG IC_{50}, μM[e]
18	2.0	85	63	7.5	>30
29	2.3	81	16	3.4	>30
33	2.1	89	15	6.4	>30
34	2.3	96	14	>10	>30

[a] Measured using shake-flask methodology with a buffer:octanol volume ratio of 100:1. The concentration of compound in the aqueous phase before and after partitioning with octanol was determined by generic HPLC-UV analysis. [b] Measured in 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] >10 μM vs 1A2, 2C19, 2C9, and 2D6. [e] Activity against the human ether-a-go-go-related gene (hERG)-encoded potassium channel was determined using conventional or automated whole-cell electrophysiology.[31]

Table 6. Phosphate Prodrugs of Compounds **18**, **29**, **33**, and **34**



No.	R	X	Y	Solubility mg/mL (Tris buffer pH 9)	Plasma concentration of drug μM[a]
37	2,3-*di*-F		OMe	25	2.6 (compound **18**)
38	2,3-*di*-F		OMe	25	0.8 (compound **29**)
39	2,3-*di*-F		H	25	3.8 (compound **33**)
5	3-F		H	25	3.0 (compound **34**)

[a] Phosphates **37**, **38**, **39**, and **5** were administered to nude mice by subcutaneous infusion using an osmotic mini-pump at 150 mg/kg/day for 48 h. Plasma concentration determined immediately at the end of the dosing period. Average plasma concentration determined from three animals.

assay.[31] The pyrazole compounds also have attractive pharmacokinetic properties displaying low to moderate clearance, following intravenous bolus injections in rats, leading to high systemic exposure *in vivo* when dosed parenterally. These four compounds also allow the *in vivo* properties of compounds with a range of Aurora A and Aurora B activities to be evaluated. While all four compounds have high activity against Aurora B kinase (Table 2 and 3), compounds **33** and **34** show much less activity against Aurora A (Table 3).

The phosphate prodrugs of compounds **18**, **29**, **33**, and **34** (compounds **37**, **38**, **39**, and **5**, respectively) (Table 6) were found to be freely soluble in 0.3 M Tris buffer at pH 9 at a concentration of 25 mg/mL. The phosphate compounds were administered as described previously,[15] at a dose of 150 mg/kg/day over a period of 48 h to nude mice bearing SW620 tumors. The plasma concentrations of the corresponding active drug compounds were measured at the end of the dosing period. As expected, each of the phosphate prodrugs generated high plasma concentrations of the corresponding active drug compounds (Table 6). Generation of the active drug substances from the corresponding phosphate prodrugs following a bolus iv-dose was also monitored so as to allow the rate and extent of conversion to be determined. For example, Figure 2 shows the mean plasma concentration of compound **5** and compound **34** at the indicated time points after a single intravenous infusion of 68 μmol/kg of compound **5**. The peak mean plasma concentrations (C_{max}) of both compound **5** and compound **34** occurred at 2 min postdose (t_{max}), indicating that compound **5** was rapidly converted into the active drug compound **34**. The plasma concentrations of compound **5** declined in a multiphasic manner following the peak concentration, and the terminal elimination half-life of compound **34** ranged from 4.9 to 5.8 h. There was no apparent difference in the plasma concentrations of compound **5** or compound **34** between the sexes (data not shown). These data, together with the data presented in Table 6, demonstrate that conversion of the phosphate prodrugs to the corresponding active drug substances is both rapid and complete following parenteral administration in rats.

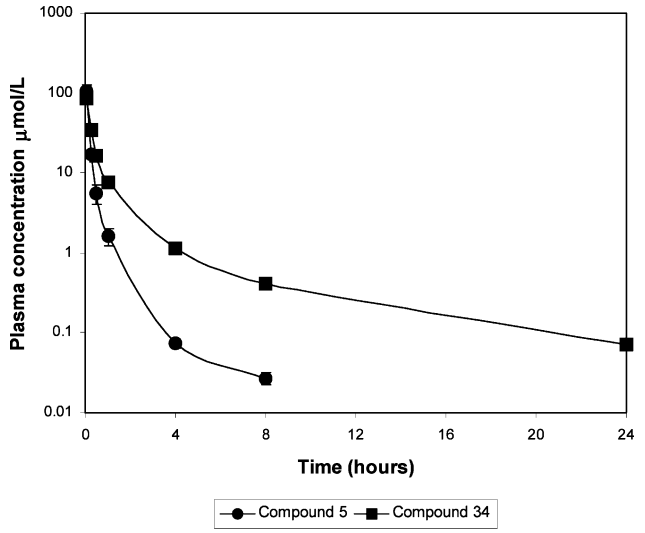


Figure 2. Group mean plasma concentrations (±SE) of compound **5** and compound **34** in male and female Wistar Hannover rats ($n = 3$ for each sex) following single intravenous bolus dosing of compound **5** at 68 μmol/kg.

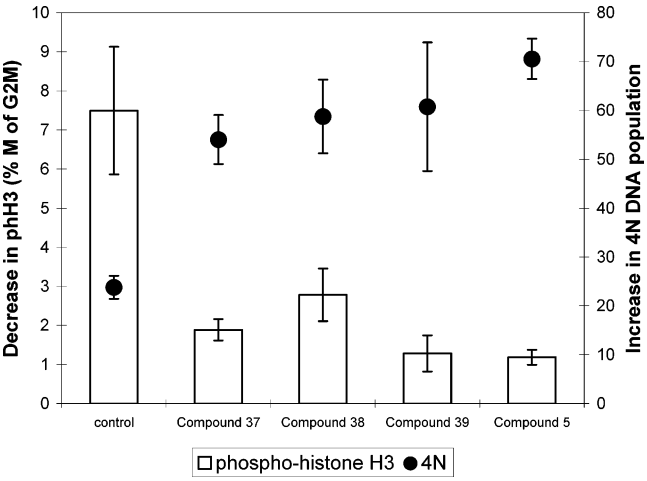


Figure 3. Pharmacodynamic activity of compounds **37**, **38**, **39**, and **5**. Human SW620 colorectal tumors were established subcutaneously in male nude mice. Compounds (150 mg/kg/day) or vehicle were administered as a constant infusion for 48 h using a subcutaneous osmotic mini-pump (implanted on the side opposite to the tumor). Following treatment the tumors were excised, disaggregated, and analyzed by flow cytometric analysis for phospho-histone H3 (PhH3) (percentage of PhH3 positive cells gated in G2/M phase of cell cycle) and 4N DNA content (percentage 4N DNA cells). Data points represent mean values from four mice per group (±SD).

All four compounds gave rise to a highly significant decrease in phosphorylation of histone-H3 in SW620 tumors (Figure 3). In all cases inhibition of histone-H3 phosphorylation correlated with an accumulation of cells with 4N DNA content compared to vehicle-treated controls. These results are fully consistent with inhibition of Aurora B kinase activity in the tumors.[1,5,23] The *in vivo* activity of the two quinazoline C-6-unsubstituted compounds **39** and **5** was particularly noteworthy with both compounds giving >75% inhibition of histone H3 phosphorylation in the tumor and up to a 3-fold increase in 4N DNA content.[23]

The robust pharmacodynamic activity of these pyrazole compounds led to their evaluation in a tumor growth inhibition study. In contrast to the earlier thiazole series, using the same

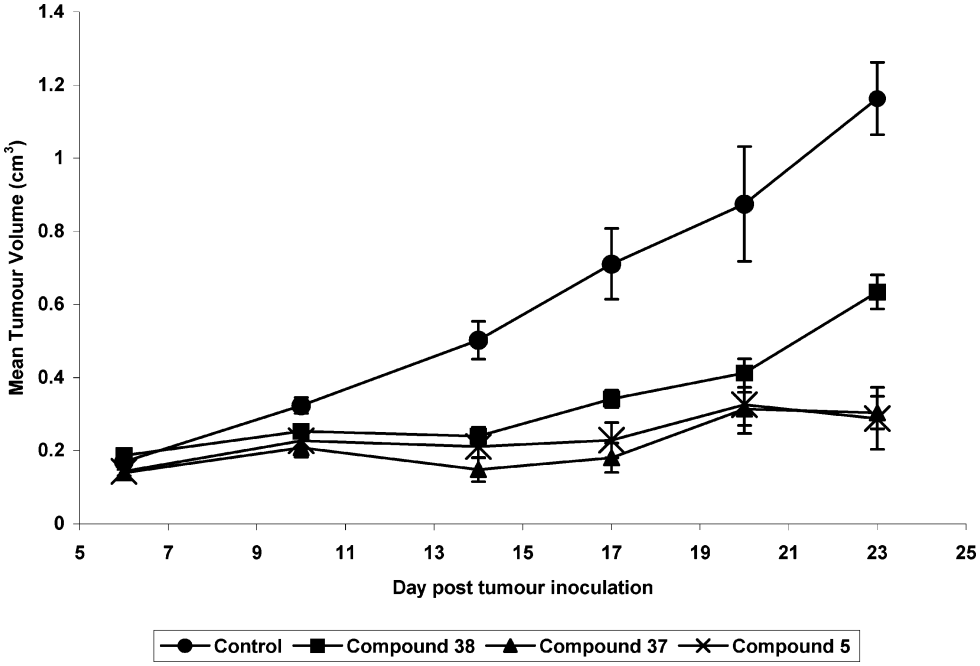


Figure 4. *In vivo* tumor growth inhibition activity of compounds **37**, **38**, and **5**. Male nude mice bearing established human SW620 colorectal tumor xenografts were dosed with either vehicle or a single cycle of compound (150 mg/kg/day) as a constant infusion for 48-h using a subcutaneous osmotic mini-pump from day 6 to day 8. Data points represent mean tumor volume (cm^3) of 10 mice (±SEM).

dosing protocol that had been employed to achieve activity against the PD marker (infusion of the compound as its phosphate derivative over a period of 48 h), compounds **37**, **38**, and **5** (compound **39** was not progressed) all demonstrated significant and durable inhibition of SW620 tumor growth in nude mice (Figure 4) at well tolerated doses. A single cycle of dosing at 150 mg/kg/day for 2 days gave rise to a reduction in tumor size of 50−87% at the end of the study (15 days after cessation of dosing) compared to tumors in vehicle-treated control animals.[23] Each of the compounds tested was well tolerated at doses where potent antitumor efficacy was observed.[23]

The pharmacodynamic effects (shown in Figure 3) for the pyrazole compounds translate into significant antitumor activity for each of the compounds tested. The highly Aurora B-selective compound **5** shows equivalent or better activity to the compounds retaining Aurora A kinase activity as would be predicted from the inhibition of histone H3 phosphorylation and percentage 4N DNA in the tumor cells (Figure 3). The striking *in vivo* activity together with the attractive biophysical and pharmacokinetic properties of the pyrazole series led us to conduct additional efficacy and safety studies. In particular, activity at lower doses using alternative dosing schedules have been explored. These data, together with activity in a number of other human tumor xenograft models, will be reported elsewhere.[23]

Conclusions

We have described a new series of pyrazole-acetanilide-substituted quinazoline Aurora kinase inhibitors that show superior *in vivo* activity over the other series that we have previously reported. The compounds show significant selectivity for Aurora B kinase, as measured in assays using recombinant enzymes, and are highly potent in cellular assays. These compounds have a novel mechanism of action (failure of cell division) that is distinct from classical antimitotics, such as Taxol, which cause cells to arrest in mitosis. The compounds show low/modest protein binding, no significant inhibition of the major P450 enzymes or the hERG ion-channel and good pharmacokinetics. When prepared as their phosphate derivatives, the compounds possess excellent solubility in simple pH-adjusted aqueous vehicles. When these phosphate prodrugs are dosed to nude mice, using subcutaneous implanted osmotic minipumps to effect continuous infusion, high exposure of the corresponding drug compounds is observed. At well-tolerated doses, inhibition of histone H3 phosphorylation is observed in SW620 tumors along with an increase in 4N DNA content and failure of cell division that leads to durable antitumor growth activity. Compound **5** has been selected for clinical evaluation and is currently in phase 1 clinical trials.

Experimental Section

General Methods. All experiments were carried out under an inert atmosphere and at room temperature unless otherwise stated. Chromatographic purifications were by flash chromatography using 40−63 μM Merck silica gel. Purification by preparative HPLC/MS was done on a Waters LC-MS system using a Waters Symmetry column (C18, 5 μM, 19 mm diameter, 100 mm length) using a mixture of water and acetonitrile with 1% TFA or acetic acid (gradient from 5% to 95%) as solvent. The purities of compounds for biological testing were assessed by analytical LC-MS on a Waters 996 photodiode array detector system using standard conditions such as the following: column, Symmetry C-18; solvent A, water 0.1% formic acid; solvent B, CH_3CN; flow rate, 2.5 mL/min; run time, 4.5 min; gradient, from 0 to 100% solvent B; mass detector, micro mass ZMD. TLCs were performed on precoated silica gel plates (Merck Art. 5715), and the resulting chromatograms were visualized under UV light at 254 nm. ^{1}H NMR spectra were recorded on an EX 400 JEOL FT or a Bruker DPX300 NMR spectrometer. Chemical shifts are reported as δ values (ppm) downfield from internal TMS in appropriate organic solutions. Peak multiplicities are expressed as follows: s, singlet; d, doublet; dd, doublet of doublet; t, triplet; br s, broad singlet; m, multiplet. Accurate mass spectra were recorded on a Micromass LCT +ve-ion electrospray spectrometer, and the results agreed with the theoretical values to within 4 ppm. NMR and mass spectra were run on isolated intermediates and final products and are consistent with the proposed structures. The combustion analyses (C, H, N) were performed with a Carlo Erba EA1108 analyzer, and the results

agreed with the theoretical values to within ±0.4%. Water was measured by the Karl Fischer method using a Mettler DL 18; HCl content was determined on a Metrohm 686 by titration using silver nitrate solution.

2-{3-[(7-{3-[4-(Hydroxymethyl)piperidin-1-yl]propoxy}-6-methoxyquinazolin-4-yl)amino]-1*H*-pyrazol-5-yl}-*N*-phenylacetamide (6). {3-[(7-{3-[4-(Hydroxymethyl)piperidin-1-yl]propoxy}-6-methoxyquinazolin-4-yl)amino]-1*H*-pyrazol-5-yl}acetic acid **43** (0.118 g, 0.25 mmol) was added to a solution of aniline (0.035 g, 0.37 mmol) in DMA (1 mL). 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.081 g, 0.43 mmol) and 2-hydroxypyridine 1-oxide (0.042 g, 0.38 mmol) were then added, and the suspension was heated at 55 °C for 2 h. The reaction mixture was purified directly by LC/MS preparative chromatography, eluting with a gradient of acetonitrile in water containing 1% acetic acid. The fractions containing product were combined and evaporated to dryness. The oily residue was dissolved in dichloromethane (with a drop of methanol), and the product was precipitated by the addition of diethyl ether. The mixture was filtered, and the residue was dried at 50 °C under vacuum overnight to yield compound **6** (0.106 g, 75% yield). ^{1}H NMR (DMSO, TFA): δ 8.94 (s, 1H), 8.29 (s, 1H), 7.63 (d, 2H), 7.31 (t, 3H), 7.05 (t, 1H), 6.83 (s, 1H), 4.27 (t, 2H), 3.99 (s, 3H), 3.82 (s, 2H), 3.60 (d, 2H), 3.30 (m, 4H), 2.97 (t, 2H), 2.27 (m, 2H), 1.89 (d, 2H), 1.65 (m, 1H), 1.44 (m, 2H). HRMS-ESI *m*/*z* 546.2829 [MH]$^+$. Anal. ($C_{29}H_{35}N_7O_4$·0.4H_2O) C, H, N, H_2O

***N*-(3-Fluorophenyl)-2-{3-[(7-{3-[4-(hydroxymethyl)piperidin-1-yl]propoxy}-6-methoxyquinazolin-4-yl)amino]-1*H*-pyrazol-5-yl}acetamide (8)**. A mixture of 2-(3-{[7-(3-chloropropoxy)-6-methoxyquinazolin-4-yl]amino}-1*H*-pyrazol-5-yl)-*N*-(3-fluorophenyl)acetamide **44** (0.136 g, 0.28 mmol) and piperidin-4-ylmethanol (0.115 g, 1.0 mmol) in DMA (1 mL) was heated at 90 °C for 8 h. The reaction mixture was purified directly by LC/MS preparative chromatography, eluting with a gradient of acetonitrile in water containing 1% acetic acid. The fractions containing product were combined and evaporated to dryness. The oily residue was dissolved in dichloromethane (with a drop of methanol), and the product was precipitated by the addition of diethyl ether. The mixture was filtered, and the residue was dried at 50 °C under vacuum overnight to give compound **8** (0.080 g, 57% yield). ^{1}H NMR (DMSO, TFA): δ 8.96 (s, 1H), 8.30 (s, 1H), 7.63 (m, 1H), 7.36 (m, 3H), 6.90 (m, 1H), 6.84 (s, 1H), 4.30 (t, 2H), 4.01 (s, 3H), 3.85 (s, 2H), 3.62 (d, 2H), 3.32 (d, 2H), 3.27 (m, 2H), 2.98 (t, 2H), 2.29 (m, 2H), 1.90 (d, 2H), 1.67 (m, 1H), 1.42 (m, 2H). HRMS-ESI *m*/*z* 564.27307 [MH]$^+$. Anal. ($C_{29}H_{34}FN_7O_4$·0.32H_2O) C, H, N, H_2O.

***N*-(2,3-Difluorophenyl)-2-{3-[(7-{3-[4-(hydroxymethyl)piperidin-1-yl]propoxy}-6-methoxyquinazolin-4-yl)amino]-1*H*-pyrazol-5-yl}acetamide (10)**. A mixture of 2-(3-{[7-(3-chloropropoxy)-6-methoxyquinazolin-4-yl]amino}-1*H*-pyrazol-5-yl)-*N*-(2,3-difluorophenyl)acetamide **45** (0.151 g 0.3 mmol), potassium iodide (0.10 g, 0.6 mmol), and piperidin-4-ylmethanol (0.115 g, 1.0 mmol) in NMP (1 mL) was heated at 60 °C for 8 h. The mixture was evaporated and the residue purified by chromatography on silica eluting with 5% methanol in dichloromethane followed by 5% methanolic ammonia in dichloromethane to give compound **10** (0.138 g, 79% yield). ^{1}H NMR (DMSO, TFA): δ 8.95 (s, 1H), 8.29 (s, 1H), 7.75 (m, 1H), 7.32 (s, 1H), 7.17 (m, 2H), 6.83 (s, 1H), 4.29 (m, 2H), 4.00 (s, 3H), 3.93 (s, 2H), 3.60 (d, 2H), 3.30 (m, 4H), 3.00 (t, 2H), 2.30 (m, 2H), 1.90 (m, 2H), 1.70 (m, 1H), 1.40 (m, 2H). HRMS-ESI *m*/*z* 582.2640 [MH]$^+$. Anal. ($C_{29}H_{33}F_2N_7O_4$·0.51H_2O) C, H, N, H_2O.

***N*-(2,3-Difluorophenyl)-2-{3-[(7-{3-[(2*R*)-2-(hydroxymethyl)-pyrrolidin-1-yl]propoxy}-6-methoxyquinazolin-4-yl)amino]-1*H*-pyrazol-5-yl}acetamide (18)**. An analogous reaction to that described for compound **10**, but starting with (2*R*)-pyrrolidin-2-ylmethanol (0.101 g, 1.0 mmol), gave compound **18** (0.134 g, 79% yield). ^{1}H NMR (DMSO, TFA): δ 8.95 (s, 1H), 8.29 (s, 1H), 7.75 (m, 1H), 7.32 (s, 1H), 7.16 (m, 2H), 6.84 (s, 1H), 4.30 (m, 2H), 4.00 (s, 3H), 3.94 (s, 2H), 3.70−3.85 (m, 1H), 3.52−3.70 (m, 4H), 3.15−3.30 (m, 2H), 2.25−2.35 (m, 2H), 1.75−2.20 (m, 4H). HRMS-ESI *m*/*z* 568.2479 [MH]$^+$. Anal. ($C_{28}H_{31}F_2N_7O_4$·1.0H_2O) C, H, N, H_2O.

***N*-(2,3-Difluorophenyl)-2-{3-[(7-{3-[(2-hydroxyethyl)(propyl)-amino]propoxy}-6-methoxyquinazolin-4-yl)amino]-1*H*-pyrazol-5-yl}acetamide (29)**. An analogous reaction to that described for compound **10**, but starting with 2-(propylamino)ethanol (0.700 g, 68 mmol) and heating at 85 °C for 5 h, gave compound **29** (0.650 g, 67% yield). ^{1}H NMR (DMSO, TFA): δ 8.96 (s, 1H), 8.30 (s, 1H), 7.75 (m, 1H), 7.33 (s, 1H), 7.18−7.22 (m, 2H), 6.84 (s, 1H), 4.30 (m, 2H), 4.00 (s, 3H), 3.94 (s, 2H), 3.78 (m, 2H), 3.30−3.45 (m, 2H), 3.28 (m, 2H), 3.15−3.20 (m, 2H), 2.28 (m, 2H), 1.73 (m, 2H), 0.95 (t, 3H). HRMS-ESI *m*/*z* 570.2640 [MH]$^+$. Anal. ($C_{28}H_{33}F_2N_7O_4$·0.4H_2O) C, H, N, H_2O.

***N*-(2,3-Difluorophenyl)-2-{3-[(7-{3-[(2*R*)-2-(hydroxymethyl)-pyrrolidin-1-yl]propoxy}quinazolin-4-yl)amino]-1*H*-pyrazol-5-yl}acetamide (33)**. An analogous reaction to that described for compound **31**, but starting with (2*R*)-pyrrolidin-2-ylmethanol (0.257 g, 2.54 mmol), gave compound **33** (0.206 g, 60% yield). ^{1}H NMR (DMSO): δ 11.60 (br s, 7H), 10.25 (s, 1H), 8.52 (m, 2H), 7.75 (m, 1H), 7.16 (m, 4H), 6.67 (s, 1H), 4.22 (t, 2H), 3.84 (s, 2H), 3.50 (d, 2H), 3.35 (m, 1H), 3.28 (m, 1H), 3.07 (m, 1H), 2.86 (m, 1H), 2.72 (m, 1H), 2.05 (m, 2H), 1.95 (m, 1H), 1.60−1.90 (m, 4H). HRMS-ESI *m*/*z* 538.2378 [MH]$^+$. Anal. ($C_{27}H_{29}F_2N_7O_3$·0.8H_2O) C, H, N, H_2O.

***N*-(3-Fluorophenyl)-2-{3-[(7-{3-[ethyl(2-hydroxyethyl)amino]-propoxy}quinazolin-4-yl)amino]-1*H*-pyrazol-5-yl}acetamide (34)**. An analogous reaction to that described for compound **31**, but starting with 2-(3-{[7-(3-chloropropoxy)quinazolin-4-yl]amino}-1*H*-pyrazol-5-yl)-*N*-(3-fluorophenyl)acetamide **48** (1.0 g, 2.2 mmol) and *N*-(ethylamino)ethanol (1.07 mL, 11.0 mmol), gave compound **34** (0.66 g, 59% yield). ^{1}H NMR (DMSO): δ 12.31 (m, 1H), 10.39 (s, 1H), 10.15 (m, 1H), 8.51 (s, 2H), 7.62 (d, 1H), 7.35 (m, 2H), 7.16 (m, 2H), 6.90 (t, 1H), 6.78 (m, 1H), 4.29 (m, 1H), 4.20 (t, 2H), 3.76 (s, 2H), 3.45 (m, 2H), 3.30 (m, 4H), 2.61 (t, 2H), 1.89 (t, 2H), 0.95 (t, 3H). HRMS-ESI *m*/*z* 508.2472 [MH]$^+$. Anal. ($C_{26}H_{30}FN_7O_3$) C, H, N.

2-[[3-({4-[(5-{2-[(2,3-Difluorophenyl)amino]-2-oxoethyl}-1*H*-pyrazol-3-yl)amino]-6-methoxyquinazolin-7-yl}oxy)propyl]-(propyl)amino]ethyl Dihydrogen Phosphate (38). (a) Di-*tert*-butyldiethylphosphoramidite (0.417 mL, 1.5 mmol) was slowly added to a solution of compound **29** (0.569 g, 1.0 mmol) in DMF (2.5 mL) in the presence of tetrazole (0.210 g, 3.0 mmol) at ambient temperature under argon. The mixture was stirred at ambient temperature for 1.5 h, and cooled to −10 °C, and hydrogen peroxide (0.134 mL of a 9.0 N solution, 1.2 mmol) was slowly added. The resulting mixture was stirred at ambient temperature for 2 h. The mixture was cooled to 0 °C, and a solution of sodium metabisulphite (0.570 g, 3.0 mmol) in water (2 mL) was added. The mixture was allowed to warm to ambient temperature and stirred for 0.5 h. The mixture was concentrated, and then a mixture of dichloromethane and methanol (8:2) was added. The mixture was filtered and the residue washed with a mixture of dichloromethane and methanol. The filtrate was concentrated *in vacuo* and the residue purified by chromatography on silica gel, eluting with a mixture of dichloromethane and methanol (90:10) and then with dichloromethane/methanol/ammonia (7.0 N) (90:10:1), to give di-*tert*-butyl 2-[[3-({4-[(5-{2-[(2,3-difluorophenyl)amino]-2-oxoethyl}-1*H*-pyrazol-3-yl)amino]-6-methoxyquinazolin-7-yl}oxy)propyl]-(propyl)amino]ethyl phosphate as an off-white solid (0.319 g, 42% yield). ^{1}H NMR (DMSO, TFA): δ 8.95 (s, 1H), 8.29 (s, 1H), 7.72 (m, 1H), 7.33 (s, 1H), 7.18 (m, 2H), 6.84 (s, 1H), 4.20−4.35 (m, 4H), 4.00 (s, 3H), 3.94 (s, 2H), 3.53 (m, 2H), 3.39 (m, 2H), 3.20 (m, 2H), 2.30 (m, 2H), 1.73 (m, 2H), 1.44 (s, 18H), 0.95 (t, 3H). MS-ESI *m*/*z* 762.5 [MH]$^+$. (b) A solution of hydrogen chloride in dioxane (4 M, 0.5 mL, 2.05 mmol) was added to a stirred suspension of compound di-*tert*-butyl 2-[[3-({4-[(5-{2-[(2,3-difluorophenyl)amino]-2-oxoethyl}-1*H*-pyrazol-3-yl)amino]-6-methoxyquinazolin-7-yl}oxy)propyl](propyl)amino]ethyl phosphate (0.316 g, 0.41 mmol) in dichloromethane (10 mL). The mixture was stirred at room temperature for 15 h and then heated to 40−45 °C for 2 h. The mixture was filtered, and the solid was dissolved in a mixture

of dichloromethane and methanol and then evaporated. The residue was triturated with dichloromethane and then dried under vacuum at 50 °C for 6 h to give compound **38** as the dihydrochloride salt (0.30 g, 100% yield). ^{1}H NMR (DMSO, TFA): δ 8.96 (s, 1H), 8.31 (s, 1H), 7.75 (m, 1H), 7.36 (s, 1H), 7.20 (m, 2H), 6.84 (s, 1H), 4.31 (t, 2H), 4.24 (m, 2H), 4.01 (s, 3H), 3.94 (s, 2H), 3.50 (m, 2H), 3.38 (m, 2H), 3.19 (m, 2H), 2.32 (m, 2H), 1.74 (m, 2H), 0.95 (t, 3H). HRMS-ESI *m*/*z* 650.22998 $[MH]^+$. (c) Compound **38** was also prepared as the free base according to the following method: compound **38**·dihydrochloride (10 g, 13 mmol) was dissolved in methanol (300 mL), and cyclohexene oxide (12.7 g, 130 mmol) was added. The solution was stirred at ambient temperature for 48 h, during which time a white solid precipitated. The mixture was diluted with diethyl ether (100 mL), and the solid was recovered by filtration, washed with ether, and dried *in vacuo* to give compound **38** as the free base (7.65 g, 88% yield) as a light yellow powder. ^{1}H NMR (DMSO, TFA): δ 8.96 (s, 1H), 8.30 (s, 1H), 7.74 (m, 1H), 7.34 (s, 1H), 7.19 (m, 2H), 6.84 (s, 1H), 4.31 (m, 2H), 4.24 (m, 2H), 4.01 (s, 3H), 3.94 (s, 2H), 3.51 (m, 2H), 3.38 (m, 2H), 3.18 (m, 2H), 2.29 (m, 2H), 1.73 (m, 2H), 0.96 (t, 3H). MS-ESI *m*/*z* 650 $[MH]^+$. Anal. ($C_{28}H_{34}F_2N_7O_7P$·1.04H_2O·0.03Et_2O) C, H, N.

{(2*R*)-1-[3-({4-[(5-{2-[(2,3-Difluorophenyl)amino]-2-oxoethyl}-1*H*-pyrazol-3-yl)amino]-6-methoxyquinazolin-7-yl}oxy)propyl]-pyrrolidin-2-yl}methyl Dihydrogen Phosphate (37). (a) An analogous reaction to that described for compound **38**, but starting with compound **18** (1.1 g, 1.9 mmol), gave di-*tert*-butyl {(2*R*)-1-[3-({4-[(5-{2-[(2,3-difluorophenyl)amino]-2-oxoethyl}-1*H*-pyrazol-3-yl)amino]-6-methoxyquinazolin-7-yl}oxy)propyl]pyrrolidin-2-yl}methyl phosphate (0.453 g, 31% yield). ^{1}H NMR (DMSO, TFA): δ 10.24 (s, 1H), 10.15 (s, 1H), 8.44 (s, 1H), 7.98 (s, 1H), 7.72 (t, 1H), 7.19 (m, 2H), 7.13 (s, 1H), 6.83 (s, 1H), 4.17 (br s, 2H), 3.93 (s, 3H), 3.85 (s, 1H), 3.77 (m, 1H), 3.56 (t, 1H), 3.54 (t, 1H), 3.08 (t, 1H), 2.94 (m, 1H), 2.66 (m, 1H), 2.47 (m, 1H), 2.20 (q, 1H), 1.94 (m, 2H), 1.86 (m, 1H), 1.69 (m, 2H), 1.60 (m, 1H), 1.37 (s, 9H), 1.36 (s, 9H). MS-ESI *m*/*z* 758.5 $[MH]^+$. (b) An analogous reaction to that described for compound **38** but starting with di-*tert*-butyl {(2*R*)-1-[3-({4-[(5-{2-[(2,3-difluorophenyl)amino]-2-oxoethyl}-1*H*-pyrazol-3-yl)amino]-6-methoxyquinazolin-7-yl}-oxy)propyl]pyrrolidin-2-yl}methyl phosphate (0.445 g, 0.59 mmol) gave compound **37** as the dihydrochloride salt (0.440 g, 94% yield). ^{1}H NMR (DMSO, TFA): δ 8.94 (s, 1H), 8.31 (s, 1H), 7.73 (m, 1H), 7.40 (s, 1H), 7.19 (m, 2H), 6.83 (s, 1H), 4.31 (t, 2H), 4.20 (m, 2H), 4.01 (s, 3H), 3.94 (s, 2H), 3.82 (m, 1H), 3.70 (m, 1H), 3.60 (m, 1H), 3.31 (m, 1H), 3.23 (m, 1H), 2.32 (m, 2H), 2.19 (m, 1H), 2.04 (m, 1H), 1.95 (m, 1H), 1.85 (m, 1H). HRMS-ESI *m*/*z* 648.21417 $[MH]^+$. (c) An analogous reaction to that described for compound **38**, but starting with the dihydrochloride salt of compound **37** (9.0 g, 0.012 mol) and recrystallizing the crude product from 20% aqueous tetrahydrofuran gave the free base of compound **37** as a pale yellow solid (6.3 g, 81% yield). ^{1}H NMR (DMSO): δ 10.30 (s, 1H), 10.20 (s, 1H), 8.50 (s, 1H), 8.00 (s, 1H), 7.70−7.80 (m, 1H), 7.20−7.30 (m, 3H), 6.70 (s, 1H), 4.30−4.40 (m, 2H), 4.10−4.20 (m, 1H), 3.90 (s, 3H), 3.80 (s, 2H), 3.70−3.75 (m, 1H), 3.40−3.50 (m, 1H), 3.30−3.35 (m, 1H), 3.20−3.25 (m, 1H), 3.05−3.15 (m, 1H), 2.90−3.00 (m, 1H), 2.10−2.20 (m, 2H), 1.90−2.00 (m, 1H), 1.70−1.80 (m, 3H). MS-ESI *m*/*z* 648 $[MH]^+$. Anal. ($C_{28}H_{32}F_2N_7O_7P$·2.3H_2O) C, H, N, H_2O.

{(2*R*)-1-[3-({4-[(5-{2-[(2,3-Difluorophenyl)amino]-2-oxoethyl}-1*H*-pyrazol-3-yl)amino]-quinazolin-7-yl}oxy)propyl]pyrrolidin-2-yl}methyl Dihydrogen Phosphate (39). (a) An analogous reaction to that described for compound **38**, but starting with compound **33** (0.437 g, 0.81 mmol), gave di(*tert*-butyl) {(2*R*)-1-[3-({4-[(5-{2-[(2,3-difluorophenyl)amino]-2-oxoethyl}-1*H*-pyrazol-3-yl)amino]-quinazolin-7-yl}oxy)propyl]pyrrolidin-2-yl}methyl phosphate (0.355 g, 60% yield) as a pale yellow solid. ^{1}H NMR (DMSO): δ 12.30 (br s, 1H), 10.20 (s, 2H), 8.50 (s, 2H), 7.68 (m, 1H), 7.10−7.20 (m, 4H), 6.78 (br s, 1H), 4.15 (t, 2H), 3.80 (m, 3H), 3.65 (m, 1H), 3.10 (m, 1H), 2.93 (m, 1H), 2.64 (m, 1H), 2.19 (m, 1H), 1.80−1.95 (m, 3H), 1.68 (m, 2H), 1.60 (m, 1H), 1.33 (s, 18H). MS-ESI *m*/*z* 730 $[MH]^+$. (b) An analogous reaction to that described for compound **38**, but starting with di(*tert*-butyl) {(2*R*)-1-[3-({4-[(5-{2-[(2,3-difluorophenyl)amino]-2-oxoethyl}-1*H*-pyrazol-3-yl)amino]-quinazolin-7-yl}oxy)propyl]pyrrolidin-2-yl}-methyl phosphate (0.355 g, 0.49 mmol), gave compound **39** as the dihydrochloride salt (0.355 g, 100% yield) as a pale yellow solid. ^{1}H NMR (DMSO): δ 12.00 (br s, 1H), 10.40 (s, 1H), 8.93 (s, 1H), 8.82 (d, 1H), 7.68 (m, 1H), 7.40−7.50 (m, 2H), 7.15−7.25 (m, 2H), 6.75 (s, 1H), 4.35 (t, 2H), 4.10−4.30 (m, 1H), 3.92 (s, 2H), 3.81 (m, 1H), 3.55−3.70 (m, 2H), 3.27 (m, 1H), 3.18 (m, 1H), 2.10−2.35 (m, 4H), 1.85−2.10 (m, 3H), 1.75−1.85 (m, 1H). HRMS-ESI *m*/*z* 618.20380 $[MH]^+$.

2-[[3-({4-[(5-{2-[(3-Fluorophenyl)amino]-2-oxoethyl}-1*H*-pyrazol-3-yl)amino]-quinazolin-7-yl}oxy)propyl](ethyl)amino]ethyl Dihydrogen Phosphate (5). (a) An analogous reaction to that described for compound **38**, but starting with compound **34** (0.62 g, 1.22 mmol), gave di(*tert*-butyl) 2-[[3-({4-[(5-{2-[(3-fluorophenyl)amino]-2-oxoethyl}-1*H*-pyrazol-3-yl)amino]-quinazolin-7-yl}oxy)-propyl](ethyl)amino]ethyl phosphate (0.539 g, 63% yield) as a pale yellow solid which was used in the next step without further characterization. (b) An analogous reaction to that described for compound **38**, but starting with di(*tert*-butyl) 2-[[3-({4-[(5-{2-[(3-fluorophenyl)amino]-2-oxoethyl}-1*H*-pyrazol-3-yl)amino]-quinazolin-7-yl}oxy)propyl](ethyl)amino]ethyl phosphate (0.539 g, 0.77 mmol), gave compound **5** as the dihydrochloride salt (0.504 g, 99% yield). ^{1}H NMR (DMSO): δ 11.98 (s, 1H), 10.79 (s, 1H), 8.93 (s, 1H), 8.83 (d, 1H), 7.65 (d, 1H), 7.47 (d, 1H), 7.38 (m, 3H), 6.89 (t, 1H), 6.74 (s, 1H), 4.32 (t, 2H), 4.28 (m, 2H), 3.85 (s, 2H), 3.42 (m, 2H), 3.34 (m, 2H), 3.27 (q, 2H), 2.29 (m, 2H), 1.28 (t, 3H). MS-ESI *m*/*z* 587.8 $[MH]^+$. (c) An analogous reaction to that described for compound **38**, but starting with the dihydrochloride salt of compound **5** (10 g, 14 mmol), yielded the free base of compound **5** as a pale yellow solid (6.54 g, 73% yield). ^{1}H NMR (DMSO): δ 10.53 (s, 1H), 8.57 (s, 1H), 8.54 (d, 1H), 7.62 (d, 1H), 7.37 (m, 2H), 7.27 (s, 1H), 7.21 (d, 1H), 6.88 (m, 1H), 6.65 (s, 1H), 4.27 (t, 2H), 4.05 (m, 2H), 3.75 (s, 2H), 3.24 (m, 2H), 3.21 (t, 2H), 3.13 (q, 2H), 2.18 (m, 2H), 1.24 (t, 3H). MS-ESI *m*/*z* 588 $[MH]^+$. Anal. ($C_{26}H_{31}FN_7O_6P$·3.0H_2O) C, H, N, H_2O.

(5-((7-(3-Chloropropoxy)-6-methoxy-quinazolin-4-yl)amino)-1*H*-pyrazol-3-yl)acetic Acid (41). A mixture of *N*′-(5-(3-chloropropoxy)-2-cyano-4-methoxyphenyl)-*N*,*N*-dimethylimidoformamide **49**[15] (14.78 g, 50 mmol) in acetic acid (40 mL) was heated at reflux with (5-amino-1*H*-pyrazol-3-yl)acetic acid **50** (8.1 g, 57.5 mmol) for 1.5 h. The reaction mixture was cooled to ambient temperature, water (250 mL) was added to the mixture, and the solid was recovered by suction filtration. The solid was washed first with water followed by ethyl acetate and finally with diethyl ether and then dried *in vacuo* at 50 °C to yield compound **41** as a yellow solid (13.6 g, 69% yield). ^{1}H NMR (DMSO, TFA): δ 8.95 (s, 1H), 8.28 (s, 1H), 7.32 (s, 1H), 6.80 (s, 1H), 4.33 (m, 2H), 4.00 (s, 3H), 3.83 (m, 2H), 3.74 (s, 2H), 2.40−2.50 (m, 2H). MS-ESI *m*/*z* 392, 394 $[MH]^+$.

(3-{[7-(3-Chloropropoxy)quinazolin-4-yl]amino}-1*H*-pyrazol-5-yl)acetic Acid (42). 4-Chloro-7-(3-chloropropoxy)quinazoline **53** (2.5 g, 9.72 mmol) and (5-amino-1*H*-pyrazol-3-yl)acetic acid **50** (1.37 g, 9.72 mmol) were combined in DMA (25 mL). A solution of 4 M HCl in dioxane (1.25 mL, 4.8 mmol) was added and the mixture heated to 90 °C for 40 min. The solution was cooled to room temperature, diluted with water (250 mL), and filtered through celite. The acidic solution was basified to pH 4.9 and the yellow powder filtered. (At pH 3, a red solid precipitated which was isolated, suspended in water, and basified to pH 12. Careful adjustment back to pH 4.8 resulted in the precipitation of a yellow powder, which was combined with the first crop). The solid was washed with diethyl ether and dried over phosphorus pentoxide to give compound **42** as a pale orange solid (2.88 g, 82% yield). ^{1}H NMR (DMSO): δ 12.60 (br s, 2H), 10.78 (br s, 1H), 8.65 (s, 1H), 8.60 (d, 1H), 7.26 (d, 1H), 7.22 (s, 1H), 6.67 (s, 1H), 4.28 (t, 2H), 3.83 (t, 2H), 3.67 (s, 2H), 2.24 (m, 2H). MS-ESI *m*/*z* 362, 364 $[MH]^+$.

{3-[(7-{3-[4-(Hydroxymethyl)piperidin-1-yl]propoxy}-6-methoxyquinazolin-4-yl)amino]-1*H*-pyrazol-5-yl}acetic Acid (43). A

mixture of (3-{[7-(3-chloropropoxy)-6-methoxyquinazolin-4-yl]-amino}-1*H*-pyrazol-5-yl)acetic acid **41** (7.83 g, 20 mmol) and 4-(hydroxymethyl)piperidine (8.05 g, 70 mmol) in DMA (30 mL) was heated at 100 °C for 2 h. The solvent was evaporated and the residue triturated with a mixture of dichloromethane and ethyl acetate (1:1). The residue was dissolved in a mixture of dichloromethane and methanol, ethanolic HCl (7.0 N) (10 mL, 70 mmol) was added, and the mixture was evaporated. Methanol (200 mL) was added to the residue, and the mixture was stirred for 0.5 h. The mixture was reduced in volume and dichloromethane added. The resultant solid was recovered by filtration and dried to yield compound **43** (6.5 g, 60% yield) as a yellow solid. ^{1}H NMR (DMSO, TFA): δ 8.96 (s, 1H), 8.31 (s, 1H), 7.37 (s, 1H), 6.80 (s, 1H), 4.31 (m, 2H), 4.00 (s, 3H), 3.75 (s, 2H), 3.59 (d, 2H), 3.24−3.30 (m, 4H), 2.97 (t, 2H), 2.35 (m, 2H), 1.86−1.91 (m, 2H), 1.68 (m, 1H), 1.47 (m, 2H).

2-(3-{[7-(3-Chloropropoxy)-6-methoxyquinazolin-4-yl]amino}-1*H*-pyrazol-5-yl)-*N*-(3-fluorophenyl)acetamide (44). (5-((7-(3-Chloropropoxy)-6-methoxyquinazolin-4-yl)amino)-1*H*-pyrazol-3-yl) acetic acid **41** (7.83 g, 20 mmol) in DMA (78 mL) was reacted with 3-fluoroaniline (2.44 g, 22 mmol) in the presence of EDC (4.2 g, 22 mmol), 2-hydroxypyridine 1-oxide (2.22 g, 20 mmol), and diisopropylethylamine (2.8 g, 22 mmol) at 50 °C for 1.7 h. The mixture was evaporated *in vacuo* and the residue triturated with water. The residue was purified by chromatography on silica gel, eluting with dichloromethane:methanol (95:3 to 85:15) to give compound **44** (4.5 g, 46% yield) as a beige solid. ^{1}H NMR (DMSO): δ 8.47 (s, 1H), 8.02 (s, 1H), 7.60−7.68 (m, 1H), 7.40 (m, 2H), 7.20−7.30 (s, 1H), 6.88 (m, 1H), 6.84 (s, 1H), 4.27 (m, 2H), 3.96 (s, 3H), 3.84 (m, 2H), 3.78 (s, 2H), 2.26 (m, 2H). MS-ESI *m*/*z* 485.6 $[MH]^+$.

2-(3-{[7-(3-Chloropropoxy)-6-methoxyquinazolin-4-yl]amino}-1*H*-pyrazol-5-yl)-*N*-(2,3-difluorophenyl)acetamide (45). 3-{[7-(3-Chloropropoxy)-6-methoxyquinazolin-4-yl]amino}-1*H*-pyrazol-5-yl)aceti c acid **41** (3.91 g, 10 mmol) was suspended in pyridine (20 mL) in the presence of 2,3-difluoroaniline (1.55 g, 12 mmol) under argon at 0 °C. Phosphorus oxychloride (1.53 g, 10 mmol) in ethyl acetate (2 mL) was slowly added at 0 °C, and the resulting mixture was allowed to warm to ambient temperature over 1.5 h. The reaction mixture was diluted with ethyl acetate (150 mL) and diethyl ether (50 mL), resulting in the precipitation of a red solid. The solid was recovered by suction filtration, dried, and resuspended in water (100 mL). The mixture was cooled to 0 °C and the pH adjusted to ∼7 by addition of 1.5 N aqueous ammonium hydroxide solution. After 15 min stirring, the solid was recovered, dried, and purified by chromatography on silica gel. Elution with dichloromethane:methanol (95/5) and then with dichloromethane:methanolic ammonia (95:2) gave compound **45** (2.55 g, 50% yield). ^{1}H NMR (DMSO, TFA): δ 8.94 (s, 1H), 8.28 (s, 1H), 7.73 (m, 1H), 7.33 (s, 1H), 7.15−7.22 (m, 1H), 6.84 (s, 1H), 4.30 (m, 2H), 4.00 (s, 3H), 3.94 (s, 2H), 3.84 (m, 2H), 2.30 (m, 2H). MS-ESI *m*/*z* 503.9 $[MH]^+$.

2-(3-{[7-(3-Chloropropoxy)-6-methoxyquinazolin-4-yl]amino}-1*H*-pyrazol-5-yl)-*N*-(3,5-difluorophenyl)acetamide (46). A suspension of 3-{[7-(3-chloropropoxy)-6-methoxyquinazolin-4-yl]-amino}-1*H*-pyrazol-5-yl)acetic acid **41** (3.91 g, 10 mmol) in DMF (20 mL) was reacted with 3,5-difluoroaniline (1.42 g, 11 mmol) in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodimide hydrochloride (2.01 g, 10.5 mmol) and 2-hydroxypyridine 1-oxide (1.11 g, 10 mmol) at 60 °C for 1.75 h. The solvent was evaporated *in vacuo*, and the residue was triturated twice with water. The resulting paste was dissolved in a mixture of dichloromethane: methanol (80:20), adsorbed onto silica gel and purified by chromatography on silica gel, eluting with dichloromethane:methanol (95:5 to 85:15) to yield compound **46** (2.45 g, 49% yield) as a beige solid. ^{1}H NMR (DMSO): δ 8.47 (s, 1H), 8.02 (s, 1H), 7.36 (m, 2H), 7.20 (s, 1H), 6.94 (t, 1H), 6.84 (s, 1H), 4.27 (m, 2H), 3.96 (s, 3H), 3.83 (m, 2H), 3.79 (s, 2H), 2.27 (m, 2H). MS-ESI *m*/*z* 503.5, 505.5 $[MH]^+$.

2-(3-{[7-(3-Chloropropoxy)quinazolin-4-yl]amino}-1*H*-pyrazol-5-yl)-*N*-(2,3-difluorophenyl)acetamide (47). 2,3-Difluoroaniline (1.15 g, 8.95 mmol) was added to a suspension of (3-{[7-(3-chloropropoxy)quinazolin-4-yl]amino}-1*H*-pyrazol-5-yl)acetic acid **42** (2.70 g, 7.46 mmol) in pyridine (30 mL) and the mixture cooled to 0 °C. Phosphorus oxychloride (1.14 g, 7.46 mmol) was added dropwise and the mixture stirred at 0 °C for 1 h. The mixture was warmed to ambient temperature, more phosphorus oxychloride (0.5 mL) was added, and then the mixture was stirred for a further 4.5 h. The mixture was diluted with ethyl acetate:ether (100 mL: 37 mL) and stirred for 18 h. The mixture was filtered and the residue suspended in water and neutralized with ammonium hydroxide (7%, 15 mL). The resultant yellow suspension was filtered, washed with water, and dried over phosphorus pentoxide to give compound **47** as an orange powder (3.15 g, 89% yield). ^{1}H NMR (DMSO): δ 10.64 (br s, 1H), 10.27 (s, 1H), 8.60 (s, 1H), 8.55 (d, 1H), 7.70 (m, 1H), 7.20 (m, 6H), 6.68 (s, 1H), 4.27 (t, 2H), 3.83 (m, 4H), 2.25 (m, 2H). MS-ESI *m*/*z* 473, 475 $[MH]^+$.

2-(3-{[7-(3-Chloropropoxy)quinazolin-4-yl]amino}-1*H*-pyrazol-5-yl)-*N*-(3-fluorophenyl)acetamide (48). Pentafluorophenyl trifluoroacetate (23.25 g, 83 mmol) was added dropwise to a solution of (3-{[7-(3-chloropropoxy)quinazolin-4-yl]amino}-1*H*-pyrazol-5-yl)acetic acid **42** (15.0 g, 41 mmol) and pyridine (6.7 mL, 83 mmol) in DMF (150 mL) with cooling to maintain the solution temperature at <23 °C. The solution was stirred at ambient temperature for 30 min before addition of 3-fluoroaniline (9.22 g, 83 mmol). The reaction was stirred for 2.5 h at ambient temperature, a further portion of 3-fluoroaniline (2 mL) was added, and the mixture was heated at 90 °C for 3 h. The reaction mixture was poured into dilute hydrochloric acid (0.1 M) and ice (ca. 500 mL), and the resultant solid was filtered, washed with water and then diethyl ether, and then dried to give compound **48** (17.7 g, 94% yield) as a brown solid. ^{1}H NMR (DMSO): δ 12.50 (br s, 1H), 10.42 (s, 1H), 8.59 (s, 1H), 8.54 (d, 1H), 7.62 (m, 1H), 7.35 (m, 2H), 7.24 (m, 1H), 7.19 (m, 1H), 6.90 (m, 1H), 6.67 (br s, 1H), 4.28 (t, 2H), 3.84 (t, 2H), 3.76 (s, 2H), 2.27 (quintet, 2H). MS-ESI *m*/*z* 455 $[MH]^+$.

7-(3-Hydroxypropoxy)quinazolin-4(3*H*)-one (52). Sodium hydride (14.6 g, 365 mmol) was added at 0 °C to a solution of 1,3-propanediol (27.8 g, 365 mmol) in DMA (70 mL). 7-Fluoroquinazolin-4(3*H*)-one **51**[21] (10 g, 60.9 mmol) was added portionwise and the reaction mixture heated at 60 °C and then at 110 °C for 3 h. The reaction was cooled to 0 °C, quenched with water (280 mL), and adjusted to pH 5.9. The resulting suspension was filtered, washed with water and then ether, and dried over phosphorus pentoxide to give compound **52** as a white powder (12.41 g, 92% yield). ^{1}H NMR (DMSO): δ 11.90 (br s, 1H), 8.04 (s, 1H), 8.00 (d, 1H), 7.10 (m, 2H), 4.17 (t, 2H), 3.58 (t, 2H), 1.92 (m, 2H). MS-ESI *m*/*z* 221 $[MH]^+$.

4-Chloro-7-(3-chloropropoxy)quinazoline (53). 7-(3-Hydroxypropoxy)quinazolin-4(3*H*)-one **52** (10.5 g, 47.7 mmol) and thionyl chloride (100 mL, 137 mmol) were combined. DMA (1 mL) was added and the reaction mixture heated to 85 °C for 1 h. The mixture was cooled to room temperature, diluted with toluene, and evaporated to dryness. This was repeated until all the thionyl chloride was removed. The residue was dissolved in dichloromethane and washed with a saturated sodium bicarbonate solution. The aqueous layer was extracted with dichloromethane. The organics were combined, dried (magnesium sulfate), and concentrated to leave a yellow solid. Trituration with diethyl ether removed a less soluble impurity, and the ether filtrate was concentrated to leave compound **53** as an off-white solid (8.5 g, 70% yield). ^{1}H NMR (DMSO): δ 13.25 (br s, 1H), 8.34 (s, 1H), 8.06 (d, 1H), 7.17 (m, 2H), 4.21 (t, 2H), 3.83 (t, 2H), 2.23 (m, 2H). MS-ESI *m*/*z* 257, 259 $[MH]^+$.

3-Amino-5-(carboxymethyl)-1*H*-pyrazole-4-carboxylic Acid (55). A suspension of 5-amino-3-(cyanomethyl)-1*H*-pyrazole-4-carbonitrile **54** (12.05 g; 82 mmol) in an aqueous solution of sodium hydroxide (10 M, 120 mL) was heated at 95→100 °C for 14 h. The resultant solution was cooled to 5→10 °C in an ice/water bath and then made acidic (pH ∼ 3) by the addition of concentrated hydrochloric acid. The resultant gray solid was removed by filtration, washed with water, and then dried in air with suction for

15 min. The residue was washed with ethyl acetate and then dried at 50 °C under vacuum for 4 h to leave compound **55** (14 g, 84% yield). ^{1}H NMR (DMSO): δ 6.5−5.25 (m, 1H), 3.55 (s, 2H).

(5-Amino-1H-pyrazol-3-yl)acetic Acid (50). 3-Amino-5-(carboxymethyl)-1*H*-pyrazole-4-carboxylic acid 0.5HCl **55** (14 g, 69 mmol) was suspended in water (280 mL), and the mixture was heated to reflux for 5 h. The mixture was allowed to cool to room temperature and then filtered. The orange-brown filtrate was evaporated to dryness, and the residue was triturated first with ethyl acetate and then with diethyl ether and then dried over phosphorus pentoxide under vacuum at 50 °C overnight to give compound **50** (10.5 g, 95% yield). ^{1}H NMR (DMSO): δ 5.88 (s, 1H), 3.70 (s, 2H).

Biological Evaluation. IC_{50} values reported are average figures of at least two independent measurements.

***In Vitro* Kinase Assays**. Drug compounds were incubated with Aurora A (Upstate Cell Signaling Solutions cat. no. 14-511) or Aurora B-INCENP and the substrate peptide. Twenty microliters of reaction mix (25 mM Tris-HCl, 12.7 mM KCl, 2.5 mM NaF, 0.6 mM dithiothreitol, 6.25 mM $MnCl_2$, 7.5μM ATP for Aurora A assay and 15 μM for Aurora B-INCENP assay, 6.25 μM peptide substrate (biotinyl-Ahx-tetra (LRRWSLG); Bachem) containing 0.2 μCi [γ^{33}P]ATP (Amersham Pharmacia, specific activity ≥ 2500 Ci/mmol)) was then added to all test wells to start the reaction (to give a final volume of 50 μL). Plates were incubated at room temperature for 60 min, reactions were stopped by addition of 100 μL of 20% v/v orthophosphoric acid, and peptide substrate was captured on positively charged nitrocellulose P30 filtermat (Whatman) using a 96-well plate harvester (TomTek) and then assayed for incorporation of ^{33}P with a Beta plate counter.

Determination of Phospho Histone H3 Suppression in SW620 Tumor Cells. Carried out as described in reference 15.

***In Vivo* Studies**. All animal experiments were conducted in full accordance with the UK Home Office Animal (Scientific Procedures) Act 1986. Male Swiss *nu/nu* mice (AstraZeneca, UK) were housed in negative pressure isolators (PFI Systems Ltd, Oxon, UK). SW620 tumor xenografts were established in 8−12-week-old mice by injecting 1×10^6 tumor cells subcutaneously (200 μL volume; in serum-free media containing 50% Matrigel; Becton Dickinson, UK) on the left dorsal flank. Animals were randomized into treatment groups when tumors became palpable. Compounds (HCl salts) were prepared in Tris buffer pH 9 and administered as a continuous infusion for up to 48 h via Alzet osmotic minipumps (Model 2001D, http://www.alzet.com/) implanted subcutaneously on the right-hand side of the animal, distal to the tumor. Tumors were measured up to three times per week with calipers, tumor volumes were calculated, and the data were plotted using the geometric mean for each group versus time. Tumor volume and tumor growth inhibition were calculated as described in reference 23. Tumor tissue was excised and frozen at the end of dosing and assessed for pharmacodynamic effects using flow cytometric analysis. Cell suspensions were prepared from the snap-frozen tumors by using an automated tissue disaggregation system (Medimachine; BD Biosystems) and fixed in 80% ethanol for a minimum of 24 h. Once fixed, disaggregated tumors were prepared for DNA content and PhH3 (ser10) assessment using a previously described staining protocol and analyzed on a FACSCalibur flow cytometer (Becton Dickinson, Franklin Lakes, NJ).[23]

Pharmacokinetic Studies. Male and female Wistar-Hannover rats (substrain crl:WI[Glx/BRL/Han]IGSBR; Charles River, UK) were multiple-housed in standard conditions until dosed. Three animals of each sex received compound **5** (68 μmol/kg) formulated in 3.3% mannitol in phosphate buffer administered intravenously (bolus injection) via a tail vein at 10 mL/kg/min by an infusion pump. Approximately 0.25 mL of blood was then collected into tubes containing anticoagulant (EDTA) from the tail vein after 2, 15, and 30 min and 1, 4, 8, and 24 h in addition to a 24 h terminal sample taken from the vena cava after administration of halothane. Plasma samples where obtained by centrifugation and transferred into separate neutral polypropylene tubes and stored frozen at or below −20 °C. Plasma samples were analyzed for compound **5** and compound **34** by solid-phase extraction followed by high-performance liquid chromatography with tandem mass spectrometric detection (HPLC-MS-MS). Pharmacokinetic analysis of the plasma concentration data was performed using the pharmacokinetic data analysis program WinNonLin Professional (Pharsight Corporation, version 3.1).

Acknowledgment. We would like to acknowledge the excellent technical expertise of the scientists at AstraZeneca, in particular, Chris Halsall for help with elemental analysis, Madeleine Vickers for mass spectra analysis, Linette Ruston for physicochemical assessment, and Chris Pollard for helpful discussion concerning hERG.

Supporting Information Available: Experimental details and data for compounds **7**, **9**, **11**−**17**, **19**−**28**, **30**−**32**, **35**, **36**; elemental analyses for all target compounds of this study. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM061335F