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Discovery of a new series of Aurora inhibitors through truncation of GSK1070916

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ABSTRACT

Novel Aurora inhibitors were identified truncating clinical candidate GSK1070916. Many of these truncated compounds retained potent activity against Aurora B with good antiproliferative activity. Mechanistic studies suggested that these compounds, depending on the substitution pattern, may or may not exert their antiproliferative effects via inhibition of Aurora B. The SAR results from this investigation will be presented with an emphasis on the impact structural changes have on the cellular phenotype.

The generation of viable chemical leads is a crucial step in the drug discovery process. The quality of a lead compound can have a profound effect on the lead-to-candidate phase of the drug discovery process, as well as on the chances that the respective candidate might be successful in the clinic.[1] Recently, fragment-based screening technologies have provided a new source of lead compounds, making fragment-based drug discovery (FBDD) an important tool in the drug discovery process.[2] One of the most important concepts in FBDD is the use of ligand efficiency indices such as BEI[3] (binding efficiency index = $pIC_{50} \times 1000/MW$), which provides a metric for assessing the quality of initial screening hits. BEI can also be used to help assess the additional contributions that new functionality makes to the overall activity of a compound as it is optimized. This concept can be applied to any drug discovery methodology, even retrospectively.[2d] The deconstruction of high molecular weight lead compounds can be used to identify the minimal core fragments with high BEI, which can serve as new starting points for lead generation.

The Aurora protein kinases (A, B, and C) are a small family of serine/threonine kinases that are expressed during mitosis and have roles in chromosome segregation and cytokinesis.[4] Because overexpression of Aurora A and Aurora B is frequently associated with tumorigenesis, these proteins have been targeted for therapy, and a number of small molecule inhibitors have been progressed to development.[4a,5] Studies have shown that mitotic defects following exposure of cells to Aurora kinase inhibitors are largely due to the inhibition of Aurora B.[6] Since Aurora B function is critical for cytokinesis, its inhibition forces the cells through a mitotic exit leading to polyploidy cells that ultimately lose viability.[7]

Recently the Aurora B inhibitor GSK1070916 was advanced as an iv agent for the treatment of cancer.[8,9] As part of our effort to find a suitable back-up series, we wanted to identify a new lead with a lower MW and higher BEI. To discover new leads, we embarked on an investigation to determine the minimum pharmacophore of GSK1070916 and use it as a fragment for optimization.

GSK1070916 evolved from compound **1**, a lead generated from cross-screening and subsequent SAR refinement (Fig. 1).[10] Since we recognized the pyrazoloazaindole 'fragment' to be present in both compounds, we started our investigation by preparing the un-substituted 4-pyrazoloazaindole **2** and the corresponding *N*-methylpyrazole derivative **3**. The synthetic route to the pyrazoloazaindoles **2** and **3** is illustrated in Scheme 1. Suzuki coupling of 4-bromoazaindole **4** with (1*H*-pyrazol-4-yl)boronic acid pinacol ester gave the corresponding phenylsulfonamide protected pyrazoloazaindole **5**. Removal of the phenylsulfonamide protecting group under basic conditions afforded 4-pyrazoloazaindole **2**. Alternatively, methylation of intermediate **5** followed by deprotection of the phenylsulfonamide group gave the *N*-methylpyrazole derivative **3**.

In addition, we prepared the truncated versions of GSK1070916 where either the arylamine group (compound **6**) or the phenylurea group (compound **9**) was removed. Compound **6** was prepared as

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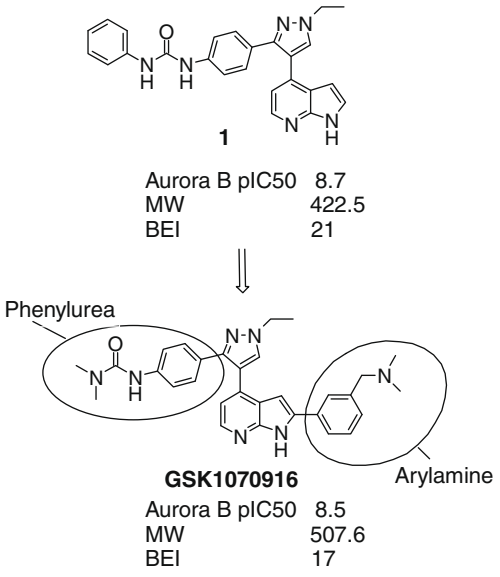
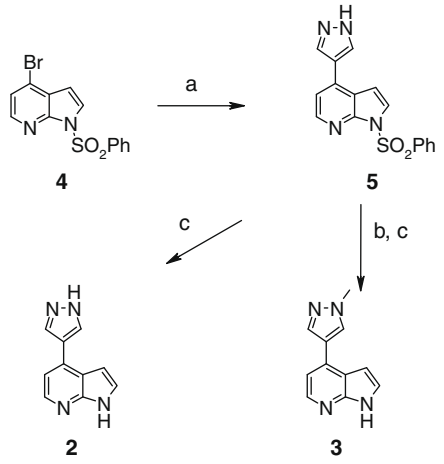
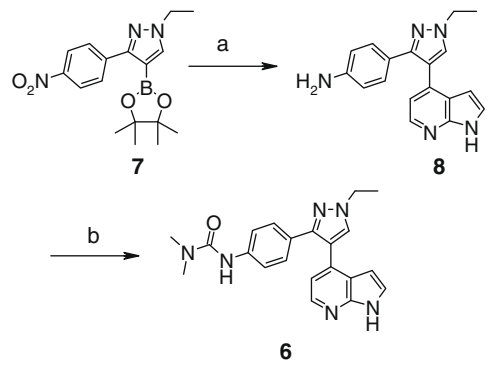


Figure 1. Discovery of GSK1070916.



Scheme 1. Reagents and conditions: (a) (1*H*-pyrazol-4-yl)boronic acid pinacol ester, $PdCl_2(PPh_3)_2$, 1,4-dioxane, aq $NaHCO_3$, 100 °C, 73%; (b) MeI, Cs_2CO_3, DMF, 72%; (c) 6 N NaOH, CH_3OH, reflux, 15% for **2**, 85% for **3**.

illustrated in Scheme 2. Suzuki coupling of *N*-ethylpyrazolyl boronic ester **7**[11] with 4-bromoazaindole followed by reduction of the nitro group gave the corresponding intermediate **8**. Reaction of aniline **8** with dimethylcarbamoyl chloride afforded compound **6**.

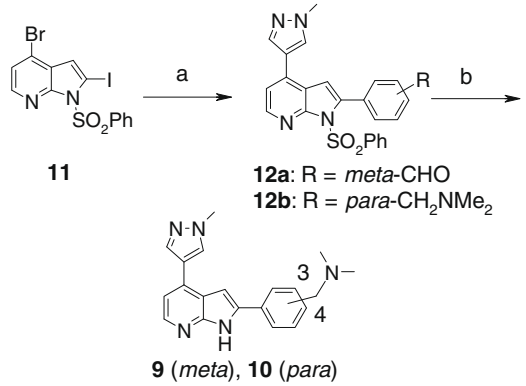


Scheme 2. Reagents and conditions: (a) (1) 4-bromoazaindole, $Pd(PPh_3)_4$, 1,4-dioxane, 2 N aq K_2CO_3, 100 °C, 80%; (2) Zn, AcOH, 85%; (b) dimethylcarbamoyl chloride, pyridine, 38%.

The syntheses of compound **9** and its respective isomer **10** were initiated by sequential Suzuki couplings of azaindole **11** with the appropriate boronic acid or ester followed by *N*-methylpyrazolyl boronic ester. Reductive amination of intermediate **12a** followed by phenylsulfonamide deprotection afforded **9**. Compound **10** was obtained from the direct deprotection of **12b** (Scheme 3).

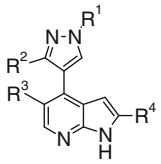
Compounds **2** and **3** showed relatively weak activity in the Aurora B enzyme assay (pIC_{50} = 6.1) but excellent binding efficiency (BEI >30), which defined the baseline level of activity of the azaindole–pyrazole template (Table 1). Compound **6**, which represents the truncated version of GSK1070916 lacking the arylamine group, exhibited a 32-fold increase in enzyme potency relative to the pyrazoloazaindoles **2** and **3**, but with a significant decrease in binding efficiency (BEI = 20). In addition, the enzyme potency did not correspond with a similar level of activity in the cell proliferation assay. Interestingly, compounds **9** and **10**, which lack the phenylurea but contained an arylamine group, both exhibited good activity in the Aurora B enzyme assay (pIC_{50} = 8.2), good binding efficiency (BEI = 25), and excellent activity in the A549 proliferation assay (Table 1). Compound **10** does show oral exposure (DNAUC = 139 ng h/mL/mg/kg) and 72% bioavailability in rats, although it has high clearance (CL_b = 86 mL/min/kg).

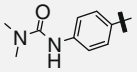
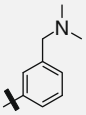
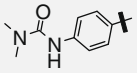
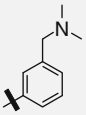
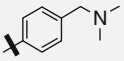
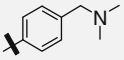
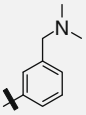
To investigate the mechanism of the antiproliferative activity, we evaluated A549 lung tumor cell lines treated with **9** and **10** using fluorescence-activated cell sorting (FACS). Given that compounds had different potencies against tumor cell growth (Table 1), in order to compare them in the FACS analysis they were all tested at 5× their EC_{50} values in the cell proliferation assay. A549 lung tumor cell lines treated with **10** showed an increase in sub-2N DNA and little or no increase in 4N/>4N DNA compared to the DMSO control (Table 2). Since inhibition of Aurora B should cause an increase in 4N/>4N DNA,[7,9] this result strongly suggested that **10** exerts its antiproliferative effects by a non-Aurora B mechanism of action. We attribute these observations to the decrease in kinase selectivity associated with the removal of the phenylurea group.[12] In contrast, FACS analysis on **9** (Table 2), exhibited an accumulation of sub-2N and 4N DNA (but not >4N DNA). Although this phenotype was not entirely consistent with that of an Aurora B inhibitor, we reasoned that **9** may be acting partially via an Aurora B mechanism of action based on the observed increase in 4N DNA.



Scheme 3. Reagents and conditions: (a) (1) $Pd(PPh_3)_4$, 1,4-dioxane, aq $NaHCO_3$, 100 °C [for **12a**: (3-formylphenyl)boronic acid; for **12b**: {4-[(dimethylamino)methyl]phenyl}boronic acid pinacol ester]; (2) (1-methyl-1*H*-pyrazol-4-yl)boronic acid pinacol ester, $Pd(PPh_3)_4$, 1,4-dioxane, aq $NaHCO_3$, 100 °C, 40% for **12a**, 45% for **12b**; (b) for **12a** to **9**: (1) Me_2NH, $NaBH(OAc)_3$, THF; (2) 6 N NaOH, CH_3OH, reflux, 26%; for **12b** to **10**: 6 N NaOH, CH_3OH, reflux, 54%.

Table 1
SAR summary



Compounds	R^1	R^2	R^3	R^4	MW	Aurora A[a] IC_{50}	Aurora B[a] IC_{50} (pIC_{50})	BEI[b]	pHH3[c,d] IC_{50}	A549[d] EC_{50}
GSK1070916	CH_3CH_2		H		507.6	1100	3.2 (8.5)	17	32	7
2	H	H	H	H	184.2	584	782 (6.1)	33	13,398	2573
3	CH_3	H	H	H	198.2	982	802 (6.1)	31	6161	11,660
6	CH_3CH_2		H	H	374.5	>10,000	26 (7.6)	20	NA	1530
9	CH_3	H	H		331.4	128	5.7 (8.2)	25	104	134
10	CH_3	H	H		331.4	76	5.9 (8.2)	25	169	24
13	CH_3	H	Cl		365.9	7.9	0.61 (9.2)	25	9	13
14	CH_3	H	Cl		365.9	6.2	0.51 (9.3)	25	67	21

[a] Values are averages of at least two experiments (nM). Experimental details describing the in vitro AurB/INCENP and AurA/TPX2 inhibition assays for IC_{50} determination are described in Ref. 8.
[b] BEI = (Aurora B $pIC_{50} \times 1000$)/MW.
[c] Cellular Mechanistic Assay. ELISA developed to measure intracellular activity of Aurora B kinase activity (nM) by determining the levels of histone-H3 (pHH3) at serine 10, a specific Aurora B substrate.
[d] Values for the cellular proliferation assay are averages of at least two experiments (nM). The percentage of human serum in the A549 proliferation assay is 10%. Further details on the cellular proliferation (A549 cells) and mechanistic pHH3 assays are described in Ref. 9. NA = not available.

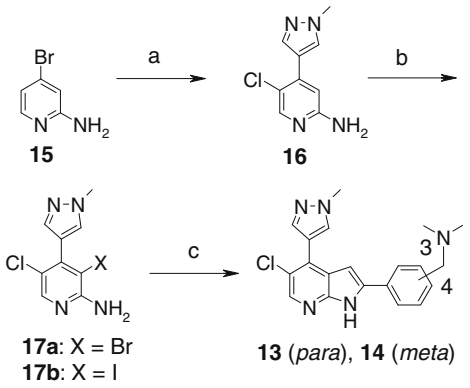
Table 2
FACS analysis in A549 cells[a]

Compounds	Concd (nM)	Sub-2N DNA	2N DNA	2N to 4N DNA	4N DNA	>4N DNA
DMSO	NA	2	63	13	20	2
GSK1070916	40	21	6	3	23	47
10	135	30	32	7	29	2
9	670	36	13	4	43	3
13	65	27	14	5	40	15
14	100	34	6	4	26	30

[a] The cell cycle profile was evaluated after 48 h. For a description of the cell cycle analysis see Ref. 9.

Since compounds **9** and **10** do not have the phenylurea moiety, we speculated that a substituent at the 5-position of the azaindole moiety could fill some space in the binding pocket and/or break the co-planarity of the pyrazole and azaindole rings to mimic more closely the conformation of GSK1070916. 5-Chloroazaindoles **13** and **14** were chosen as our first targets to test the viability of our strategy. Their synthesis is described in Scheme 4. Chlorination of position-5 of 4-bromo-2-aminopyridine, followed by Suzuki cross-coupling with *N*-methylpyrazole boronic ester, afforded the aminopyridine intermediate **16**. Halogenation at the 3 position with NBS in DMF at room temperature, or ICl in DMF at 40 °C, gave intermediates **17a** and **17b**, respectively. Sonogashira cross-coupling with the corresponding alkyne, followed by base induced cyclization generated the desired chloroazaindole compounds **13** and **14**.[13]

Gratifyingly, both compounds **13** and **14** exhibited high potency in the Aurora B enzyme, phosphorylation of histone-H3, and A549 proliferation assays (Table 1). FACS analysis demonstrated that **13** causes an increase in 4N/>4N DNA (40/15%) compared to the DMSO control, suggestive of an Aurora B mode of action (Table 2). In addition, multipolar spindles were observed at 12 nM and 37 nM by immunofluorescent microscopy, also consistent with an Aurora B mode of action (Fig. 2). Compound **14**, which combines the 5-chloroazaindole hinge binder and the *meta*-aminomethyl group in the tail region, has a phenotype more consistent with Aurora B inhibition. FACS analysis of **14** exhibited the highest



Scheme 4. Reagents and conditions: (a) (1) NCS, DMF, 53%; (2) (1-methyl-1*H*-pyrazol-4-yl)boronic acid pinacol ester, $Pd(PPh_3)_2Cl_2$, 1,4-dioxane, aq $NaHCO_3$, 100 °C, 45%; (b) for **17a**: NBS, DMF, 52%; for **17b**: ICl, DMF, 40 °C, 78%; (c) (1) Zn, NaI, DMSO, Et_3N, DBU, $Pd(PPh_3)_4$, 80 °C [for **13**: {4-[(dimethylamino)methyl]phenyl}ethyne; for **14**: {3-[(dimethylamino)methyl]phenyl}ethyne]; (2) KO*t*-Bu, DMF, 6% for **13** from **17a**, 25% for **14** from **17b**.

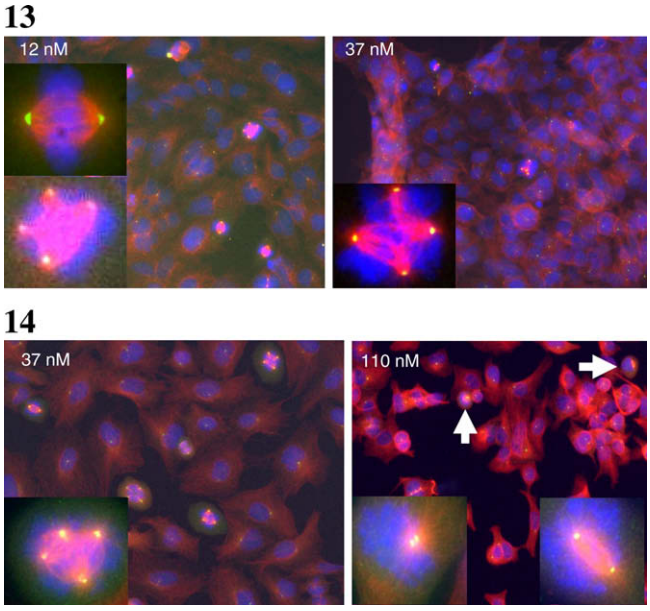


Figure 2. Mitotic phenotype of **13-** and **14**-treated A549 tumor cells by immunofluorescent staining. A549 cells were treated with 12, 37, or 110 nM of **13** and **14** for 24 h and stained with α-tubulin antibody (red staining) to visualize mitotic spindles, pericentrin antibodies for centrosomes (yellow staining), and 4′,6-diamino-2-phenylindole for DNA (blue staining).

accumulation of >4N DNA (30%) of the four compounds, and was similar to GSK1070916 (Table 2). In addition, analysis by immunofluorescent microscopy showed multipolar spindles at 37 nM, and a mixture of malformed bipolar spindles (phenotype associated with Aurora B inhibition) and monopolar spindles (phenotype associated with Aurora A inhibition) at 110 nM (Fig. 2).

Compared to GSK1070916, compound **14** has similar Aurora B enzyme and cellular activity, and exhibits a cellular phenotype consistent with Aurora B inhibition. Since compound **14** has a relatively low MW (365) and high binding efficiency (BEI = 25), it offers a valuable starting point for the development of another class of Aurora B inhibitors. This work illustrates the value of deconstructing and reconstructing an advanced compound to generate new, more ligand efficient leads for further optimization.

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