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Synthesis and evaluation of alkenyl indazoles as selective Aurora kinase inhibitors

Stéphanie Blanchard *, Anthony D. William, Angeline C.-H. Lee, Anders Poulsen, Ee Ling Teo, Weiping Deng, Noah Tu, Evelyn Tan, Kay Lin Goh, Wai Chung Ong, Chee Pang Ng, Kee Chuan Goh, Zahid Bonday, Eric T. Sun

S*BIO Pte. Ltd, 1 Science Park Road, #05-09 The Capricorn, Singapore Science Park II, Singapore 117528, Singapore

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ABSTRACT

A series of alkenyl indazoles were synthesized and evaluated in Aurora kinase enzyme assays. Several promising leads were optimized for selectivity towards Aurora B. Excellent binding affinity and good selectivity were achieved with optimized compounds in isolated Aurora subfamily assays.

Cancer cells typically contain mutations in a number of genes, which ultimately result in uncontrolled cell growth and tumor metastasis. As enzymes specific for and essential to cell growth and division, Aurora kinases hold the potential to be important control points for slowing the growth and spread of tumors. Aurora family kinases regulate important events during mitosis including centrosome maturation and separation, mitotic spindle assembly, and chromosome segregation. Misregulation of Aurora kinases due to genetic amplification and protein overexpression results in aneuploidy and may contribute to tumorigenesis.

Three Aurora kinase isoforms A, B and C are a family of serine–threonine kinases that are believed to play multiple roles in the development and progression of cancer, by acting as regulators of cell proliferation, by transforming normal cells into cancer cells and by down-regulating the tumor suppressor p53. The Aurora enzymes are expressed in mammals, each of which is thought to play important roles in regulating mitosis. Overexpression of Aurora A leads to centrosome amplification and polyploidy. Aurora B is known to phosphorylate histone H3 during mitosis, which may play a role in chromosome condensation. Both Aurora A and B are over-expressed in many tumor types, including colon cancer, breast cancer and leukemia. Aurora B has been described as the more suitable anticancer target because its inhibition results in catastrophic mitosis that leads to cell death.[1–3] Validation in mouse xenograft studies[4,5] supports the concept of targeting Aurora kinases with small molecule inhibitors as a potentially powerful approach to new antitumor therapies. Moreover advanced programs in several companies have led to new small molecule Aurora inhibitors recently entering the clinic.[2]

As part of our program towards the development of anticancer kinase inhibitors, we have developed indazoles with Aurora kinase subfamily selectivity.

Our in-house compound library was screened for inhibitors of Aurora A and B resulting in the discovery of compound **1** (Fig. 1) as a potent Aurora A inhibitor with an IC_{50} of 0.21 μM. The subsequent hit to lead process was heavily influenced by prior art in the area of kinase inhibitors and led to the design of new molecules based on indazole moiety **2**.

Herein we disclose the optimization strategy applied to the indazole scaffold leading to potent and selective Aurora B inhibitors.

Introduction of various benzimidazole replacements at the indazole 5-position (R^2) were initially investigated. A styryl group (double bond spacer, see compounds **5**, **6** and **7**) was identified as the preferred linker over alkyne or cyclopropyl linking moieties for

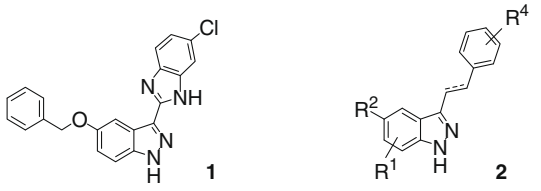

Figure 1.

* Corresponding author. Tel.: +65 68275050.
E-mail address: stephanie_blanchard@sbio.com (S. Blanchard).

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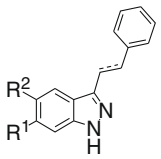
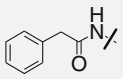
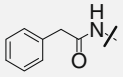
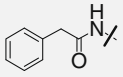
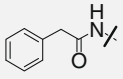
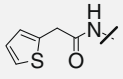
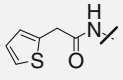
Table 1

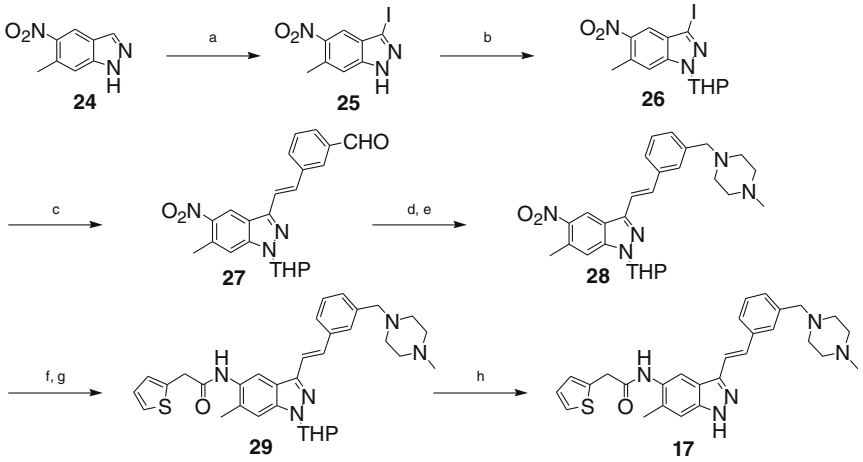


Compound	R^2	R^1		Aur A IC_{50}[9] (μM)	Aur B IC_{50}[10] (μM)
3		H		1.7	–
4		H		0.64	1.64
5		H		0.022	–
6		Me		0.019	–
7		Me		0.017	0.047
8		Me		0.24	0.11

Aurora A activity, with a 10-fold improvement in potency over **1** (Table 1). Substitution of the indazole 6-position (R^1) was well tolerated and conferred novelty. To guide further optimization studies, models of the Aurora A and B ATP binding sites were utilized from published crystal structures[7,8] enabling new compounds to be docked yielding much information from the subsequent analysis. These computational studies showed that the linker should be flat to avoid a clash with the protein (clash seen with alkyl and cyclopropyl linkers). Although the alkyne linker is flat, it showed less hydrophobic contacts than the double bond.[6]

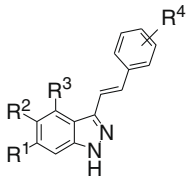
Alkene substituted indazoles were easily accessible using a high yielding procedure; the synthesis of representative Aurora inhibitor **17** is outlined in Scheme 1. After iodination of 6-methyl-5-nitro-indazole (**24** prepared according to the literature[11]) and protection of the N1 position, Heck reaction followed by reductive amination were carried out to give nitro compound **28**. Then **28** was reduced to the corresponding amine which was reacted with thiophen-2-yl-acetyl chloride to yield, after THP deprotection, alkenyl indazole **17**. Compounds **9**–**15** were prepared using a corresponding chloro-nitroindazole and the cyclic amines were introduced by chloride-displacement under basic conditions.[11]

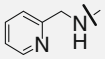
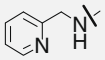
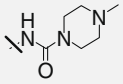
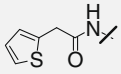
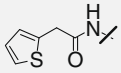
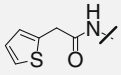
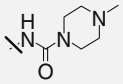
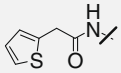
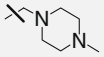
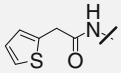
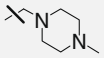
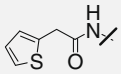
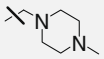
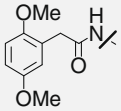
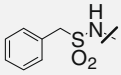
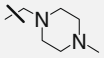
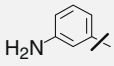
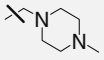
Our first goal was to optimize general potency by exploring three key substitution positions: R^1, R^2 and R^3 (Table 2). Target compounds were chosen based on docking and in silico analysis of drug-like properties such as *c*Log *P* and polar surface area. Potent compounds in the mono-substituted series, $R^1 = R^3 = H$ (**18** and **21**)



Scheme 1. Synthesis of Aurora inhibitor **17**. Reagents and conditions: (a) I_2, KOH, DMF (98%); (b) DHP, pTSA, CAN (98%); (c) 3-vinyl-benzaldehyde, $Pd(OAc)_2$, P(*o*-tolyl)$_3$, Et_3N, DMF, 100 °C (70%); (d) 1-methyl-piperazine, DCM; (e) $Na(OAc)_3BH$ (71%); (f) $SnCl_2{\cdot}H_2O$, DCM/MeOH; (g) thiophen-2-yl-acetyl chloride, pyridine, DCM (55%); (h) 4 N HCl in dioxane, MeOH (69%).

Table 2
Activity and selectivity of indazole Aurora inhibitors



Entry	R^2	R^1	R^3	R^4	Aur A IC_{50}[9] (μM)	Aur B IC_{50}[10] (μM)	Selectivity A/B
9			H	H	0.16	0.046	3.5
10			H	H	>10	0.81	>12
11			H	H	0.62	0.13	5
12	NO_2		H	H	0.24	0.11	2.2
13	NH_2		H	H	0.20	0.081	2.5
14			H		0.14	0.062	2.3
15			H	H	0.091	0.11	0.8
7		Me	H	H	0.017	0.047	0.36
16		Me	H		0.013	0.046	0.28
17		Me	H		0.079	0.13	0.6
18		H	H		0.035	0.026	1.3
19		H	Me		1.9	0.099	**19**
20		Me	H	H	0.042	0.18	0.23
21		H	H	H	0.035	0.031	1.1
22	CN	H	Me		0.28	0.059	4.7
23		H	Me		0.35	0.031	11.3

gave 30 nM IC_{50s} for both Aurora A and B. Although these compounds were potent they were neither novel nor selective. To address these issues di-substitution on the indazole ring was investigated firstly with the assessment of the effect of the size of the 6-position group, R^1. Various groups such as alkyl or cyclic amines were introduced demonstrating that small to medium sized lipophilic groups were preferred for potency (examples **7**, **11** and **15**) whereas larger groups such as methyl-piperazine (**10**) showed a decrease in activity due to a clash with the Val147 residue in the active site.

Examining the role of R^2, analogues containing a nitro or amine function suffered a decrease in potency (**11**, **12** and **13**) for Aurora A and B but, encouragingly, exhibited some selectivity (2–5-fold) towards Aurora B. Installation of an amide or sulfonamide moiety boosted potency to below 100 nM against one or both isozymes (**7**, **14**, **20** and **21**).

In the course of our SAR studies, exploration of R^3 provided a breakthrough in the quest for selectivity towards Aurora B. Switching the methyl group from R^1 (**17**) to R^3 (**19**) dramatically improves selectivity (24-fold swing) towards Aurora B. Similar results were seen with compounds **22** and **23**. A docking study (**19**) showed that the methyl group at this position changes the conformation of the amide linker creating a clash with the Aurora A protein explaining the selectivity towards Aurora B and the dramatic potency loss to-

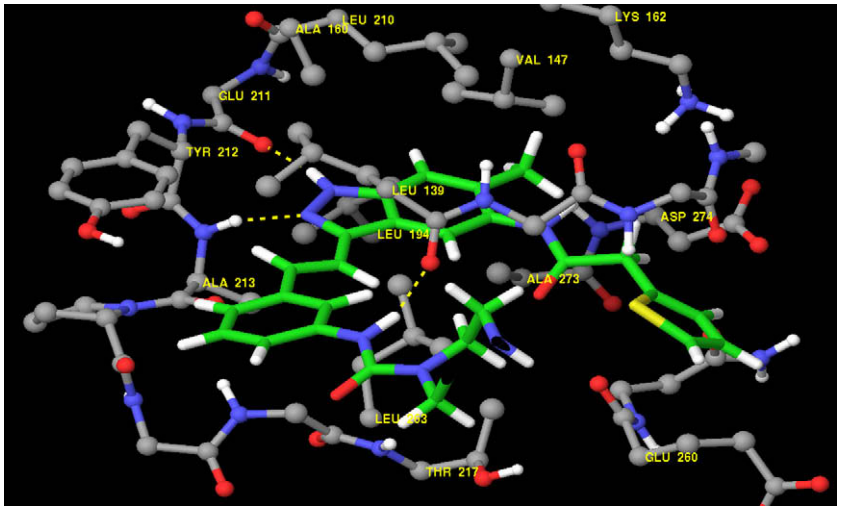


Figure 2A. Compound **16** docked into the ATP binding site of the Aurora A X-ray structure 1MQ4. Compound **16** is displayed in tube representation with green carbons. Aurora A is displayed in wire representation with gray carbons. The alpha carbons are labeled with three letter residue name and number. Hydrogen bonds between **16** and Aurora A are displayed with yellow dashed lines. Glu211 and Ala213 are the same hinge region residues which hydrogen bond to the purine group in ATP.

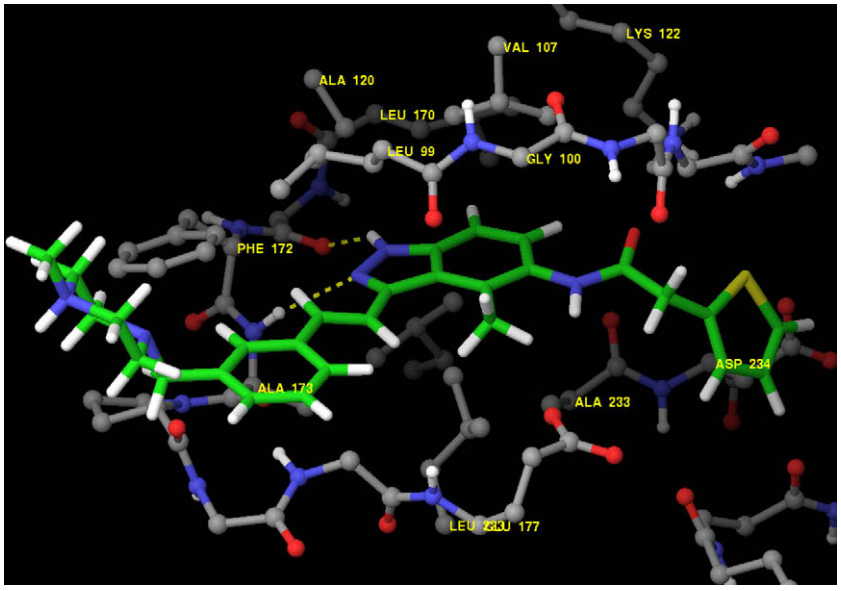


Figure 2B. Compound **19** docked into the ATP binding site of the Aurora B X-ray structure 2BFX. Due to the 4-methyl substituent the amide of the 5-position is rotated 180° compared to **16** docked into Aurora A. This orientation of the amide substituent can only be docked into Aurora B in a low energy conformation.

wards Aurora A[6] (Fig. 2B). A comparison of Aurora A and B X-ray structures revealed that the binding sites only differ by two residues. Tyr212 and Thr217 in Aurora A correspond to Phe172 and Glu177, respectively in Aurora B[6].

In parallel we investigated the right hand side of the molecule with an assessment of R^4. Introduction of hydrophilic groups (**14**, **16**, **17** and **18**) lowers *c*Log *P* while maintaining good potency. Docking of **16** showed additional hydrogen bonds to the Aurora A protein besides the two important interactions from the indazole moiety itself to the hinge region (Fig. 2A). The compounds were equipotent against both enzymes.

In conclusion we have designed and synthesized a series of alkenyl indazoles with potent in vitro inhibition of both Aurora A and B in the low nanomolar range. Furthermore, it has been shown that small differences in active site architecture can be exploited to achieve a significant degree of selectivity of one isozyme over the other. Our studies culminated in the discovery of **19** which had potent Aurora B activity and nearly 20-fold selectivity over Aurora A.

Although these compounds were potent and drug like with good cell penetration properties we were unable to demonstrate good cellular inhibition. Further work is needed to progress this series into more advanced anticancer studies.

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9. *Aurora A assay*: The recombinant Aurora A kinase is a hexahistidine-tagged full-length protein expressed in *Hi-5* insect cells using the expression plasmid pFBHTb (Invitrogen, USA). The activity assay uses the PKLight™ HTS Protein Kinase Assay Kit from Cambrex Corporation (New Jersey, USA). This assay measures the consumption of ATP and is based on the bioluminescent detection of the ATP remaining in the wells after the kinase reaction. The reaction mixture consisted of 10–15 nM of recombinant kinase and 5.0 μM of ATP (its K_m value) in 40 μL assay buffer (25 mM Hepes pH 7.5, 25 mM KCl, 5 mM $MgCl_2$, 5 mM b-glycerophosphate, 1 mM dithiothreitol). The affinity-purified kinase undergoes robust autophosphorylation and no exogenous substrate is added. The compounds were tested at eight concentrations prepared from fourfold serial dilution starting at 10 μM. The reaction was incubated at room temperature for 2 h. Twenty microliters of PKLight™ ATP detection reagent was added and the reaction was incubated for another 10 min. Luminescence signals were detected on a multi-label plate reader (Victor^2 V 1420, Perkin–Elmer). IC_{50} values were generated from the raw data using the Prism 4.0 software (GraphPad Software Pte Ltd). All IC_{50} values are the mean from at least two independent experiments.
10. *Aurora B assay*: The recombinant Aurora B kinase is a GST-tagged full-length protein expressed in *Hi-5* cells using the expression plasmid pFB-GST (Invitrogen, USA). The activity assay is based on the principles of time-resolved fluorescence (TRF). The reaction mixture consisted of 20–40 nM of enzyme, 0.5 μM of biotinylated substrate (Cell Signaling Technology, Inc. (CST) Cat#1300) and 40 μM of ATP (its K_m value) in 50 μL assay buffer (CST Kinase Buffer Cat# 9802). The compounds were tested at eight concentrations prepared from fourfold serial dilution starting at 10 μM. The reaction was incubated at room temperature for 1 h. Twenty-five microliters of STOP buffer (60 mM Hepes pH 7.5, 60 mM EDTA) added and 30 μL quenched mixture was transferred to Neutravidin coated plates (Cat#15402B, Pierce). After incubation at room temperature (rt) for 30 min, the plates were washed with 4 × 80 μL Tris-buffered saline with 0.5% Tween-20 (TBST). About 50 μL of antibody mixture (CST antibody Cat# 5070 and Perkin–Elmer antibody Cat# AD0124) was added and the reaction incubated for 30 min, followed by washing with 4 × 80 μL TBST. About 50 μL of Dissociation-enhanced lanthanide fluorescence immunoassay (DELFIA) Enhancement Solution (Perkin–Elmer Cat#4001-0010) was added and the reaction incubated for 5 min. TRF signals were detected on a multi-label plate reader (Victor^2 V 1420, Perkin–Elmer). IC_{50} values were generated from the raw data using the Prism 4.0 software (GraphPad Software Pte Ltd). All IC_{50} values are the mean from at least two independent experiments.
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