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Discovery of a potent and selective Aurora kinase inhibitor

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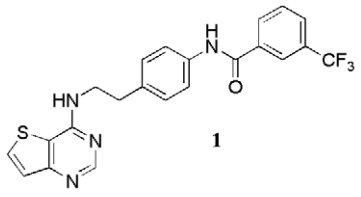
ABSTRACT

This communication describes the discovery of a novel series of Aurora kinase inhibitors. Key SAR and critical binding elements are discussed. Some of the more advanced analogues potently inhibit cellular proliferation and induce phenotypes consistent with Aurora kinase inhibition. In particular, compound **21** (SNS-314) is a potent and selective Aurora kinase inhibitor that exhibits significant activity in pre-clinical in vivo tumor models.

The Aurora family of serine/threonine kinases are key regulators of mitosis.[1,2] There are three human isoforms (Aurora A, B, and C) all of which have been strongly implicated in the onset and propagation of cancer.[3,4] For example, Aurora A is a putative oncogene,[3,5,6] and is over-expressed in a number of human malignancies including colon, breast, pancreatic, and ovarian tumors.[4] Recent clinical success with kinase inhibitors in oncology has prompted much interest in Aurora kinases as potential targets for small-molecule intervention.[7–10] A few Aurora kinase inhibitors are already undergoing clinical trials.[3,4,6,10–16] Herein, we report our medicinal chemistry efforts that started with a low micromolar hit (**1**, Fig. 1) and led to the selection of a potent and selective Aurora kinase inhibitor (SNS-314) as a candidate for clinical development.[17]

In-house screening identified 2-aminoethyl phenyl benzamide derivative **1** as a low micromolar inhibitor of Aurora A (Fig. 1). The compound has modest molecular weight and was shown to have acceptable in vitro metabolic stability and good cellular permeability. Screening against a larger panel of kinases (Upstate) indicated that **1** is a fairly selective inhibitor of Aurora A, leading to its selection for optimization.

The general synthesis of key analogues is outlined in Scheme 1.[17] Reacting 2-aminothiazole derivative **2**[18] with Boc-anhydride



Aurora A, IC_{50} = 3200 nM
mLM (% @ 0.5h): 40
hLM (% @ 0.5h): 42
MDCK: 31 x 10^{-6} cm/s
(efflux ratio: 1.1)
M_w: 442 g/mol

Figure 1. Low micromolar Aurora A inhibitor that served as a starting point for medicinal chemistry optimization.

followed by removal of the phthaloyl group using hydrazine yielded mono-protected bis-amine **3** in 76% overall yield. Subsequent S_NAr displacement employing 4-chloro-thienopyrimidine in the presence of Hunig's base followed by Boc-deprotection under acidic conditions afforded intermediate **4** in moderate yield (66%). Finally, urea formation was achieved by heating **4** in the presence of various isocyanates to give the desired inhibitors in variable yields. Alternatively, converting **4** into the corresponding phenyl-carbamate derivative followed by reaction with various amines afforded urea analogues in poor to good yields.

Initial structure–activity studies attempted to replace the 3-trifluoromethylphenyl ring of **1**, but failed to yield significant improvements in activity (data not shown). We therefore focused next on variations in the linker region (Table 1). Reversing the amide linkage in **1** caused a complete loss of activity (**5**), whereas the corresponding urea (**6**) displays activity similar to **1**. Moving

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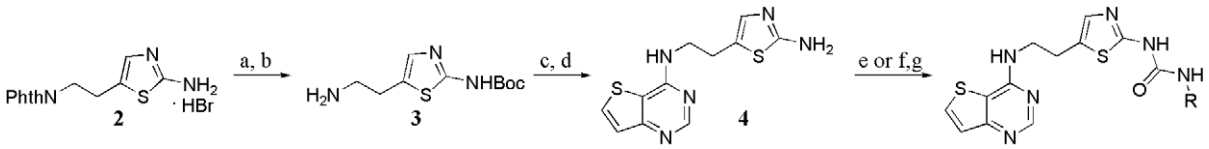
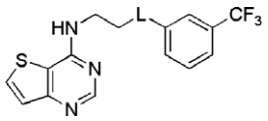
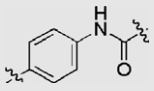
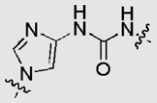
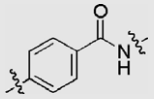
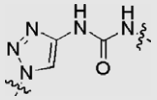
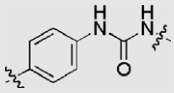
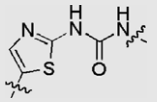
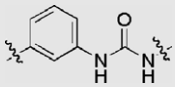
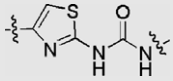


Scheme 1. Reagents and conditions: (a) Boc_2O, DMAP, NEt_3, MeCN, reflux, 86%; (b) H_2NNH_2, THF, 65 °C, 88%; (c) 4-chlorothieno[3,2-*d*]pyrimidine, DIEA, DMF, 90 °C, 75%; (d) 4 M HCl/Dioxane, 88%; (e) RNCO, Toluene, 90 °C, 20–80%; (f) PhOCOCl, NEt_3, THF, 0 °C to rt, 80%; (g) RNH_2, cat. DMAP, 80 °C, DMSO, 10–80%.

Table 1
Initial SAR of the linker moiety of **1**[a]



Compound	L	Aurora A IC_{50} [nM]	Compound	L	Aurora A IC_{50} [nM]
1		3200	**8**		1200
5		>20,000	**9**		250
6		1200	**10**		22
7		>20,000	**11**		>20,000

[a] Typically average of at least two dose–response curves. Variation generally <100%. See Supplementary data and Ref. 17 for assay protocols.

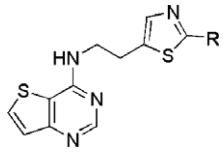
the urea linkage from the 4 to the 3 position of the central aryl ring abolishes biochemical activity (**7**), suggesting specific engagement with the protein. By contrast, replacing the central ring with 3-substituted imidazole is tolerated (**8**), and the corresponding triazole analogue is about 10-fold more active than **1** (**9**, Table 1). Finally, introduction of a 2-aminothiazole moiety as the central ring results in an even more active compound, **10**, with an IC_{50} against Aurora A in the low nanomolar range. Interestingly, the regioisomeric 2-aminothiazole derivative **11** does not show any appreciable activity.

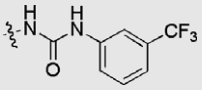
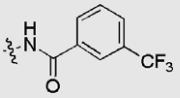
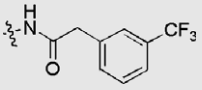
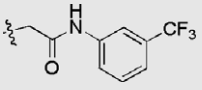
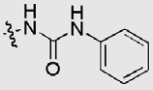
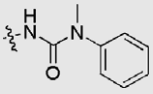
Compound **10** was also shown to be a strong inhibitor of Aurora B (IC_{50} = 14 nM, Table 2). Consistent with other pan-Aurora kinase inhibitors,[6,12,19,20] the cellular profile of compound **10** is dominated by its inhibition of Aurora B. Compound **10** decreases phosphorylation of the Aurora B substrate histone H3 (pHH3) in cell culture (HCT116, Fig. 2A) and also causes endoreduplication (appearance of 8N DNA) in mitotic cells at low nanomolar concentrations (Fig. 2B). Furthermore, the compound inhibits cellular proliferation of the HCT116 cell line with an EC_{50} of 13 nM as measured by incorporation of BrdU (Table 2).

Having verified the cellular phenotype, we next explored the importance of the urea moiety in **10** (Table 2). Replacing the urea with an amide results in significantly reduced activity (cf. **10** and **12**), as does *N*-methylation (cf. **15** relative to **16**), in both biochemical and cellular assays (proliferation and cell-cycle analysis). Replacing either NH moiety of the urea with a methylene also abrogates activity (**10** vs **13** or **14**, Table 2). To further understand the importance of the urea and the binding mode of compound **10**, an X-ray crystal structure of **10** complexed with humanized mouse Aurora A was solved (Fig. 3, PDB code: 3d14). Inhibitor **10** binds the kinase in the catalytic cleft and forms a hydrogen bond between the thienopyrimidine N1 and the main chain NH of Ala226 in the hinge region (Fig. 3A). The bi-aryl urea portion of the compound extends deep into the selectivity pocket of the protein.[21] Interestingly, although the compound extends toward the α-C helix, the DFG loop of Aurora A is in a conformation typically associated with an activated state.[21] The crystal structure also explains the importance of the urea moiety, which is involved in an intricate hydrogen-bond network with the protein (Fig. 3B). The urea oxygen is within close proximity (2.9 Å) of the catalytic lysine (Lys175), while the two NH groups form a bidentate hydrogen-bond interaction (3.0 and 2.9 Å) with the catalytic glutamic acid residue (Glu194). This network is consistent with the structure–activity relationships in Table 2. In addition, the endocyclic thiazole nitrogen forms a hydrogen bond with a water molecule which in turn also coordinates the glutamic acid (Glu194) as well as the primary amide moiety of glutamine 198 (Fig. 3B).

Understanding the importance of the 2-aminothiazole urea linker, we next turned our attention to modifications of the aniline portion of the inhibitors (Table 3). Removal of the 3-trifluoromethyl substituent results in reduced biochemical- and cellular activity (cf. compounds **15** and **10**). Other 3-substituents are generally tolerated (e.g., **17–21**), with the 3-chloro analogue **21** being equipotent to **10**. Moving the chloro-substituent from the 3 to the 4 position results in modest loss of activity (**21** vs **22**), whereas the corresponding 2-substituted derivative is significantly less active (**21** vs **23**). Di-substituted analogues, for example, compounds **24** and **25**, generally show modest to good activity, but do not offer any advantages over the mono-3 substituted versions. Heterocyclic right-hand side moieties were also made and tested. For example,

Table 2
SAR of the urea moiety in compound **10**[a]



Compound	R	Aurora A IC_{50} [nM]	Aurora B IC_{50} [nM]	BrdU[b] EC_{50} [nM]	FACS[c] EC_{50} [nM]
10		22	14	13	<16
12		2500	NT	NT	1000–4000
13		>10,000	580	>10,000	NT
14		4900	442	NT	NT
15		52	120	45	16-63
16		7200	NT	>10,000	NT

[a] See Ref. 17 and Supplementary data for assay protocols.
[b] Cellular proliferation as determined by BrdU incorporation.
[c] Cell-cycle profile as determined by FACS analysis performed with 16, 63, 250, and 1000 nM concentrations of compound, cf. Figure 2B. EC_{50}s reported as a range when ⩾50% of the DNA content is 4 N or higher.

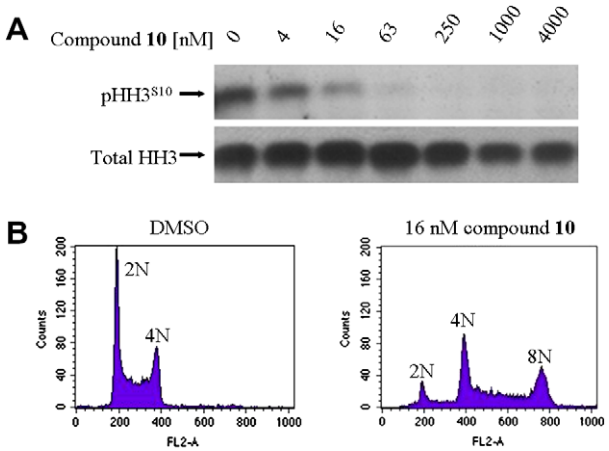


Figure 2. Cellular characterization of compound **10**. See Ref. 17 for experimental details. (A) Western blot analysis of cell-extracts from HCT116 cells treated with compound **10** for 16 h. (B) FACS analysis of HCT116 cells treated with 16 nM compound **10** for 16 h.

indole-derivative **26** has good activity in both the biochemical and the cellular assays. Alkyl-derived ureas were also examined, such as compound **27**. However, unsaturated analogues were found to be less potent in general (e.g., **27**, Table 3) and also tended to be metabolically labile. We also evaluated compounds containing amines. As expected, incorporation of an amine results in significantly improved aqueous solubility at lower pH. Some amine containing analogues also display modest to good activities (e.g., **28** and **29**, Table 3), but unfortunately they tended to be heavily efflluxed and have less favorable in vivo pharmacokinetic profiles as well (data not shown).

As mentioned above, modulation of histone H3 phosphorylation is a useful biomarker for the assessment of Aurora kinase inhibitors, and has been used in pre-clinical as well as clinical studies.[19] In initial PK/PD/activity assessments, we observed that anti-tumor activity in xenograft models required extended suppression of histone H3 phosphorylation in tumor tissue. Compounds with good overall profiles were therefore further evaluated for their ability to decrease the levels of pHH3 in HCT116 cell culture and in in vivo HCT116 xenograft models (Target Modulation, Table 4) using Western blot analysis (cf. Fig. 2).[17] Generally, efficient reduction of pHH3 levels in vivo requires both strong in vitro suppression of pHH3 and high compound levels in the tumor. For example, compound **20** strongly inhibits the formation of pHH3 in vitro but only exhibits significant target modulation at the higher dose (100 mg/kg, ip), correlating well with the amount of inhibitor present in the tumor (Table 4). Halogen-substituted analogues, including **21** and **25**, usually show high tumor concentrations and considerable target modulation. Interestingly, although indole-derived **26** has modest cellular activity and yields relatively low tumor concentrations, it surprisingly shows significant in vivo suppression of pHH3. In contrast, amine containing analogues (e.g., **28** and **29**) typically show lower tumor concentration and also exhibit modest cellular

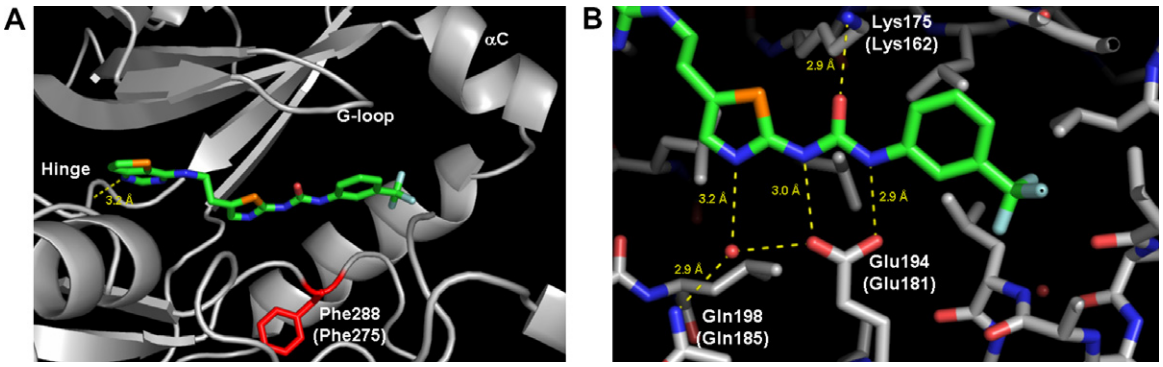
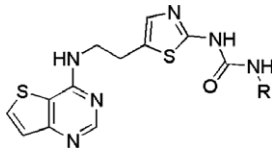
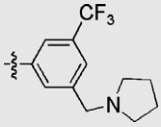
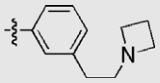


Figure 3. Co-crystal structure of compound **10** (green) complexed to humanized mouse Aurora A, see the Supplementary data for details. Numbering based on mouse Aurora A with the corresponding human numbers shown in parentheses. (A) Compound **10** binds Aurora A in the DFG-in conformation and makes a hydrogen bond with the hinge. Phe288 (in the DFG triad) is highlighted in red. (B) Hydrogen-bonding network of the 2-aminothiazole urea moiety; see text for details. Molecular images were produced with PYMOL (DeLano, W. L. (2004) *PyMOL*, http://pymol.sourceforge.net/).

Table 3
SAR of the urea substitution[a]



Compound	R	Aurora A IC_{50} [nM]	Aurora B IC_{50} [nM]	BrdU[b] EC_{50} [nM]	FACS[c] EC_{50} [nM]
10	3-CF_3-C_6H_4	22	14	13	<16
15	-Ph	52	120	45	16–63
17	3-Me-C_6H_4	9	54	19	<16
18	3-MeO-C_6H_4	50	NT	34	16–63
19	3-CCH-C_6H_4	41	NT	14	<16
20	3-F-C_6H_4	23	18	6	<16
21	3-Cl-C_6H_4	9	31	6	<16
22	4-Cl-C_6H_4	39	NT	30	16–63
23	2-Cl-C_6H_4	280	NT	376	>250
24	3,4-Cl_2-C_6H_3	96	13	24	16–63
25	3-Cl-4-F-C_6H_3	11	51	5	<16
26	7-Indolyl	30	19	31	16–63
27	Cyclohexyl	270	61	72	63–250
28		4	22	6	<16
29		53	120	121	16–63

[a] See Ref. 17 and Supplementary data for assay protocols.
[b] Cellular proliferation as determined by BrdU incorporation.
[c] Cell-cycle profile as determined by FACS analysis performed with 16, 63, 250, and 1000 nM concentrations of compound, cf. Figure 2B. EC_{50}s reported as a range when ⩾50% of the DNA content is 4 N or higher.

(pHH3) activity. In accordance, they tend to display poor to modest target modulation (Table 4).

Among the compounds evaluated in target modulation studies, analogues **10**, **21**, and **26** had the most promising overall profiles and were selected for head-to-head comparison in xenograft anti-tumor models and toxicology studies. Although all three analogues showed good activity in multiple models, analogue **21** (SNS-314) was ultimately selected for development based on its tolerability under multiple dosing schedules and ease of manufacturing (data not shown).

A co-crystal structure of SNS-314 complexed with Aurora A indicates that the compound has the same binding mode as analogue **10**, and the protein adopts the DFG-in conformation (see Supplementary data). The hydrogen bonding interactions of the urea are also conserved.

The selectivity profile of SNS-314 was determined against a panel of 219 kinases (Upstate, Table 5). In this set, only 7 kinases were found to be inhibited with IC_{50}s within 100-fold of Aurora A. In addition, SNS-314 induces a cellular phenotype consistent with Aurora kinase inhibition and exhibits potent anti-proliferative activity in a diverse panel of human cancer cell lines.[22] The compound also shows significant in vivo anti-tumor activity in a number of pre-clinical xenograft models. For example, intermittent dosing (150 mg/kg ip, biwx3) results in 96% tumor growth inhibition (day 36) in an HCT116 mouse xenograft model.[22]

Table 4
In vitro and in vivo target modulation: suppression of pHH3[a]

Compound	Aurora B IC_{50} [nM]	pHH3[b] EC_{50} [nM]	Target modulation score:[c] in vivo inhibition of pHH3			
			50 mg/kg (ip)		100 mg/kg (ip)	
			Score at 6 h (Tumor conc.)[d]	Score at 10 h (Tumor conc.)[d]	Score at 6 h (Tumor conc.)[d]	Score at 10 h (Tumor conc.)[d]
10	14	<16	4(NA)	3(NA)	4(NA)	4(NA)
20	11	<16	2(1.20)	1(<1.00)	4(101)	2(19.8)
21	31	<16	4(9.07)	3(3.72)	4(28.6)	4(31.6)
24	13	16–63	1(21.3)	1(12.5)	2(49.5)	2(54.8)
25	51	<16	4(25.8)	2(15.6)	4(112)	3(56.1)
26	19	16–63	4(4.82)	4(3.67)	4(16.9)	4(4.36)
28	22	16–63	2(NA)	1(NA)	3(6.20)	2(8.76)
29	120	63–250	1(<1.00)	1(<1.00)	NA	NA

[a] See Ref. 17 for experimental details.
[b] In vitro suppression of pHH3 (S10) as determined by Western blot analysis performed with 16, 63, 250, and 1000 nM concentrations of compound using the HCT116 cell line. EC_{50}s reported as a range, cf. Figure 2A.
[c] In vivo suppression of pHH3 (S10) as evaluated by densitometry of Western blots, cf. Figure 2A. Mouse HCT116 xenograft model was used. Score 1: 0–25% inhibition, score 2: 25–50% inhibition, score 3: 50–75% inhibition, score 4: 75–100% inhibition.
[d] Amount of compound [μM] detected in the tumor at the indicated time.

Table 5
Kinase selectivity profile of SNS-314 (**21**)[a]

Kinase	IC_{50} [nM]
Aurora	<1.0
Trk B	5.0
Trk A	12
Flt4	14
Fms	15
DDR2	82
Axl	84
c-Raf	100

[a] See text for details. Selectivity against 219 kinases determined at Upstate at the respective ATP Km of each kinase (radiometric assay).

In conclusion, we have disclosed a novel series of Aurora kinase inhibitors. Key SAR as well as crucial binding elements have been described. Further, we have shown that the more advanced analogues have potent activities in cell-based assays and induce phenotypes consistent with Aurora kinase inhibition. Moreover, these profiles translate into efficient target modulation (pHH3) in vivo. In particular, analogue **21** (SNS-314) is a potent and selective Aurora kinase inhibitor that displays significant activity in pre-clinical in vivo models. The compound is currently in clinical trials in patients with advanced solid tumors.

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Supplementary data

Co-crystal structure of SNS-314 and Aurora A (PDB code: 3d15); procedures for crystallization and structure determination; crystallographic data; Aurora A kinase assay protocol. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.07.073.

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