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Novel series of pyrrolotriazine analogs as highly potent pan-Aurora kinase inhibitors

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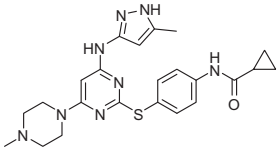
ABSTRACT

The synthesis and SAR for a novel series of pyrrolotriazines as pan-Aurora kinase inhibitors are described. Optimization of the cyclopropane carboxamide terminus of lead compound **1** resulted in analogs with high cellular activity and improved rat PK profiles. Notably, compound **17l** demonstrated tumor growth inhibition in a mouse xenograft model.

Aurora kinases (Aurora A, B, C), a family of serine/threonine kinases, play a key role in regulating cell mitosis. Aurora A, ubiquitously expressed, regulates mitotic entry and centrosome maturation.[1] Aurora B, also widely distributed, is essential for chromatin remodeling and cytokinesis. It is also known to phosphorylate histone H3 (HH3) at Ser-10 during mitosis.[2] Aurora C's function during mitosis is much less well defined, and it is suggested to have an overlapping role with Aurora B.[3] Accordingly, inhibition of Aurora kinases results in cell cycle disruption and cell death. Both Aurora A and B gene amplification and protein over-expression have been frequently detected in a variety of tumors. Thus, disruption of overly active Aurora function is expected to impair tumor growth and has the potential for broad antitumor activity in a number of cancers.[4]

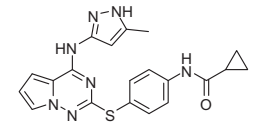
Based on these premises, the Aurora kinases have been actively pursued as targets for the discovery of new cancer chemotherapeutics.[5] As a result, several small molecule inhibitors of Aurora kinases have been identified, some of which have reached clinical evaluation, including Aurora-A selective MLN8054,[6] Aurora-B selective AZD1152[7] and pan-Aurora inhibitor VX-680 (MK-0457)[8] (Fig. 1). However, the ideal inhibitor profile for therapeutic use in relation to the Aurora isoform selectivity has not yet been defined clinically.

Compound **1** (Fig. 1), a novel lead molecule based on the *N*-(1*H*-pyrazol-3-yl)pyrrolo[2,1-*f*][1,2,4]triazin-4-amine scaffold, was disclosed as an ATP-competitive, pan-Aurora inhibitor, with moderate activity in inhibiting pHH3 formation and proliferation in HCT-116 cells.[9] Herein we report the structure–activity relationship



VX-680 (MK-0457)

K_ds = 4, 7, 6 nM (Aurora A, B, C)
IC_{50} = 55 nM (HCT-116 pHH3)
IC_{50} = 28 nM (HCT-116 proliferation)



1

K_ds = 12, 20, 4 nM (Aurora A, B, C)
IC_{50} = 431 nM (HCT-116 pHH3)
IC_{50} = 345 nM (HCT-116 proliferation)

Figure 1. Literature pan-Aurora inhibitor and internal lead compound (Ambit internal data).

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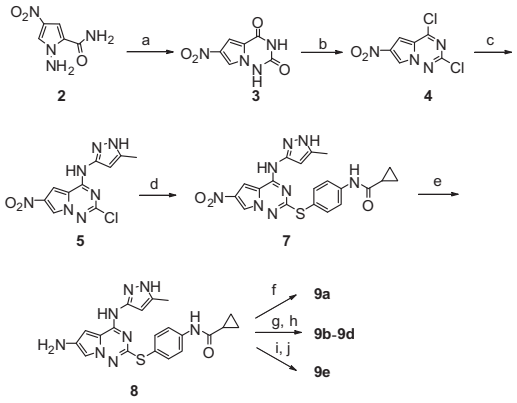


Scheme 1. Reagents and conditions: (a) ethyl chloroformate, pyridine, dioxane, 125 °C, 72%; (b) $POCl_3$, TEA, reflux, 125 °C, 37%; (c) 5-methyl-1*H*-pyrazol-3-amine, KI, DIEA, DMF, rt, 46%; (d) *N*-(4-mercaptophenyl)cyclopropanecarboxamide (**6**), K_2CO_3, DMF, 120 °C, 12 h, 39%; (e) Raney Ni, H_2, MeOH; (f) EDC, HOBT, DIEA, DMF, rt, 3-(piperidin-1-yl)propanoic acid, 54% over two steps; (g) $(BrCH_2CO)_2O$, THF, 83% over two steps; (h) amines, rt, ca. 67%; (i) 4-nitrophenyl carbonochloridate, THF; (j) 1-methylpiperazine, TEA, THF, 8% over three steps.

(SAR) development of this series of compounds aimed at producing highly potent analogs with desirable PK profiles by using various basic groups as formal solubilizing groups in different regions of the molecule. Such a strategy has been widely applied in kinase inhibitor design to improve the cellular activity and solubility of resulting inhibitors. It is also compatible with parenteral administration, which is desired for minimizing any potential gastrointestinal toxicity associated with orally dosed pan-Aurora inhibitors.

SAR studies began with modifications at the 6-position of pyrrolotriazine, due to synthetic accessibility and a favorable vector for reaching a solvent exposed space from this position. Synthesis of such compounds started from the nitro amino pyrrole **2**,[10] which was converted to the dione **3** (Scheme 1). Chlorination of **3** using $POCl_3$ followed by regioselective displacement of the 4-chloro group in **4** with aminopyrrazole afforded compound **5**. Nucleophilic substitution of the 2-chloro in **5** with benzenethiol **6** provided the amide **7**. The nitro group of **7** was reduced using Raney nickel without affecting the sulfide bond to give a somewhat labile amino compound **8**. Subsequent elaboration of the amino group as shown in Scheme 1 yielded analogs **9a–9e**.

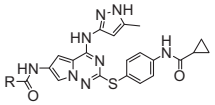
As shown in Table 1, among the aminoalkyl amides prepared (**9a–9d**), only the morpholino- (**9b**) and D-prolinol- (**9d**) analogs achieved similar binding affinity[11] to compound **1**, while piperidinyl analog **9a** and morpholino analog **9b** exhibited slightly improved cell activity in inhibiting HCT-116 cell proliferation. The urea analog **9e** was found to have equivalent binding affinity for Aurora kinases, and two to fourfold improvement in cellular activity. However, **9e** suffered high clearance in a screening rat PK study, compared to **1** (Table 5).

The compounds described herein were also monitored for their kinase selectivity in a panel of 317 kinases (290 distinct kinases plus the mutants) using the KinomeScan technology. In most cases, the kinome selectivity dropped off with the introduction of basic amines, reflected by the increase in selectivity score[12] compared to lead compound **1** (S_{10} score of 0.03 vs a panel of 202 distinct kinases). Such shift of kinome selectivity usually implies additional interactions with non-Aurora kinases elicited by the additional 6-position substituents.

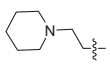
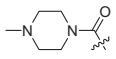
SAR attention was then turned to the modification of the cyclopropane carboxamide portion of the molecule via functionalizing the amide terminus. X-ray structure of **1** in complex with Aurora A suggests that the cyclopropane carboxamide points to the back specificity pocket (data not shown). Hydrophilic (basic) groups have been successfully introduced in this region of the protein in kinase inhibitor design, exemplified by the successful discovery of imatinib.[13] Synthesis of these compounds was made possible by acylation of compound **10**[9] with either chloroacetyl chloride or bromoacetic anhydride, followed by straightforward displacement of the halides with various amines to give **12a–12e** (Scheme 2).

SAR for some representative aminoacetamides is shown in Table 2. No boost in the potency was observed with most of analogs (**12a–12d**), regardless of the amines attached at the α-position of the acetamide. Compound **12e**, an acetamide with (*S*)-pyrrolidin-2-ylmethanol at the α-position, exhibited marginally improved cell potency and moderate kinome selectivity. The presence of an alcohol group in **12e** should in principle be favorable in solubilizing the compound and in providing a handle for further functionalization. Compound **12e** did suggest that the

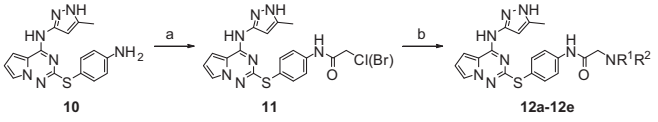
Table 1
SAR of substitution on 6-amino pyrrolotriazines for Aurora kinases



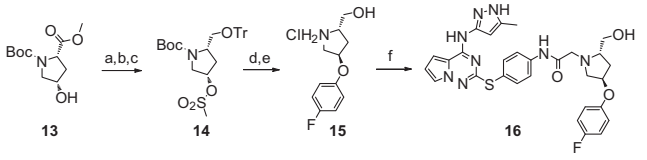
9a-9e

Entry	R	Aurora K_d[a] (nM)			Cell IC_{50}[a] (nM)		Kinase specificity score S(10)
		A	B	C	pHH3 (HCT-116)	Proliferation (HCT-116)	
9a		38	37	10	850	101	0.27
9b		7	9	3	420	160	0.12
9c		23	49	13	828	536	0.08
9d		10	16	80	6300	536	0.34
9e		7	4	5	190	75	0.31

[a] Each experiment was run in duplicate and the values shown are the average of the two.



Scheme 2. Reagents and conditions: (a) chloroacetyl chloride, THF, 0 °C–rt, 97%; or bromoacetic anhydride, THF, −15 °C to rt, 76%; (b) HNR^1R^2, KI, DIEA, DMF, 70 °C, 10–44%.



Scheme 3. Reagents and conditions: (a) $LiBH_4$, THF, 80 °C, 5 h, (b) Tr-Cl, Pyr. rt, 4 d, 61% over two steps; (c) MsCl, TEA, DCM, rt, 99%; (d) *p*-fluorophenol, DMF, microwave, 30 min, 120 °C, 77%; (e) 4 N HCl in Dioxane, rt; (f) **11**, KI, DIEA, DMF, 70 °C, 3 h, 33%.

α-aminoacetamide side chain could be a productive area for further SAR exploration.

Encouraged by the results of **12e**, the effect of additional substitution at the 4-position of the pyrrolidine ring was examined. Scheme 3 describes one representative example of requisite analog preparation. Hydroxyproline carboxylate **13** was reduced and the resulting primary alcohol was selectively capped as its trityl ether, and the secondary alcohol was mesylated to give **14**. Inversion of the stereochemistry by SN2 substitution of the mesylate with a phenol, followed by simultaneous deprotection of acid labile groups revealed pyrrolidinol **15**, which reacted with chloride **11** to afford analog **16**. Syntheses of other prolinol derivatives shown in Table 3 can be found in Supplementary data.

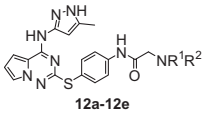
Introduction of a *trans*-*t*-butoxy group at the 4-position resulted in **16a**, which exhibited high binding affinity for Aurora kinases and excellent cell activity in the HCT-116 proliferation assay (IC_{50} = 8 nM) (Table 3). Consistent with potent inhibition of Aurora B, **16a** also inhibited the histone H3 phosphorylation in HCT-116 cells with an IC_{50} value of 15 nM and exhibited good kinome selectivity with an S10 score of 0.12. Further expansion of the pyrrolidine 4-position SAR ensued. A variety of liphophilic groups were well tolerated, including difluoro and both diastereoisomers of mono-fluoro pyrrolidine analogs (**16b–16d**), along with the large *p*-fluorophenoxy substituent (**16f**). Quite interestingly, the cyclopropane-fused pyrrolidine analog (**16e**) was less well tolerated, as was *t*-butylthioether **16h**. Introduction of polar groups such as hydroxyl (**16g**) or amino (**16j**) greatly reduced the potency. The prolinol stereochemistry at 2-position is critical for the Aurora inhibitory activity, since epimerization of this stereocenter (**16g**) led to more than 100-fold loss of cell potency.

The rat PK profile of compound **16a** showed high clearance and low AUC following iv dosing (Table 5). It was hypothesized that the primary hydroxyl group might be a cause for the high clearance. We decided empirically to switch the 2-hydroxymethyl group to the 3-hydroxy group to keep the distance between the hydroxyl group and the pyrrolidine nitrogen constant. This maneuver resulted in 3,4-*trans*-disubstituted pyrrolidine compound **17a**, which recapitulated the Aurora binding affinities and cellular potency of **16a**, with somewhat decreased kinome selectivity (Table 4). The in vivo clearance and exposure of **17a** in rat iv PK are only marginally better than those of **16a** (Table 5). Undeterred by this single data point, more 3,4-disubstituted pyrrolidine analogs were prepared and characterized.

The synthesis of 3,4-*trans*-disubstituted pyrrolidines mostly originated from the appropriately protected 2,5-dihydro-1*H*-pyrrole, such as **18**, which could be converted to epoxide **19**, as shown in Scheme 4. Epoxide opening with various alcohols and subsequent deprotection yielded the requisite pyrrolidine derivatives **20**. Alkylation with chloroacetamide **11** yielded the desired analogs **17**. Additional manipulations of 2,5-dihydro-1*H*-pyrroles to access other pyrrolidines are described in Supplementary data.

Similar to the 2,4-disubstituted pyrrolidine analogs, hydrophobic groups at the 4-position of 3,4-*trans*-disubstituted pyrrolidine are well tolerated, including variations of the *t*-butyl group, such as **17c–17d**, **17f–17h** (Table 4). Fluoro-substituted (**17b**) and methoxyethoxy-substitued analogs (**17e**) fared worse in the HCT-116 proliferation assay. Compound **17i** demonstrated that the 3-hydroxyl group is not absolutely required; an oxime group at the same position is almost as potent as **17a**. The phenoxy group in compound **16f** was also explored in the context of 3,4-disubstituted pyrrolidine. *p*-Fluorophenoxy analog **17l** is fivefold more potent than **16f**, and the regiochemistry of the fluorine substitution (**17j–17k**) had no effect on cell activity. 2,4- and 3,5-disubstituted phenoxy analogs (**17m–17n**) are essentially equipotent to **17l**.

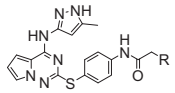
Table 2
SAR of aminoacetamide analogs for Aurora kinases



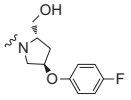
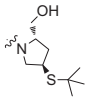
Entry	NR^1R^2	Aurora K_d[a] (nM)			Cell IC_{50}[a] (nM)		Kinase specificity S10
		A	B	C	pHH3 (HCT-116)	Proliferation (HCT-116)	
12a		32	41	6	288	364	0.25
12b		143	42	14	655	365	0.16
12c		85	41	17	1830	1840	0.10
12d		31	8	5	293	370	0.14
12e		14	4	1	146	148	0.20

[a] Each experiment was run in duplicate and the values shown are the average of the two.

Table 3
SAR of pyrrolidinoacetamide analogs for Aurora kinases

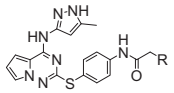


16a-16j

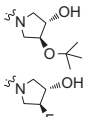
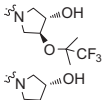
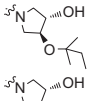
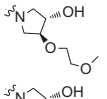
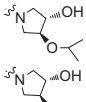
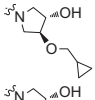
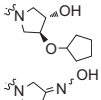
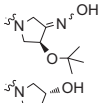
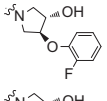
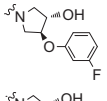
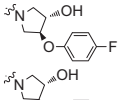
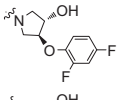
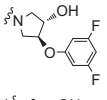
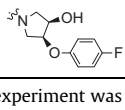
Entry	R[c]	Aurora K_d[a] (nM)			Cell IC_{50}[a] (nM)		Kinase specificity score S(10)
		A	B	C	pHH3 (HCT-116)	Proliferation (HCT-116)	
16a		9	8	2	15	8	0.12
16b		8	5	2	29	25	0.28
16c		15	9	ND[b]	91	104	0.20
16d		8	4	ND	28	25	0.23
16e		17	15	ND	179	203	0.18
16f		53	21	ND	78	24	0.15
16g		301	69	ND	2490	2390	0.007
16h		111	39	ND	1790	1610	0.08
16i		58	15	ND	637	596	0.115
16j		381	144	ND	15,000	4700	0.103

[a] Each experiment was run in duplicate and the values shown are the average of the two.
[b] ND = not determined.
[c] Single enantiomer.

Table 4
SAR of pyrrolidinoacetamide analogs for Aurora kinases



17a-17o

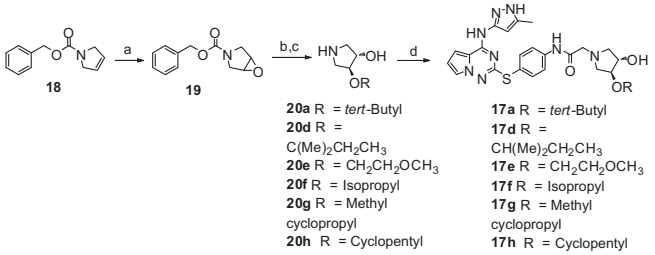
Entry	R[c]	Aurora K_d[a] (nM)			Cell IC_{50}[a] (nM)		Kinase specificity score S(10)
		A	B	C	pHH3 (HCT-116)	Proliferation (HCT-116)	
17a		12	5	ND[b]	16	4	0.22
17b		5	1	ND	47	33	0.21
17c		6	4	ND	27	9	0.27
17d		8	4	ND	28	9	0.21
17e		5	2	ND	49	25	0.22
17f		8	4	ND	23	10	0.20
17g		7	2	ND	23	10	0.27
17h		11	4	ND	23	6	0.26
17i[d]		9	5	ND	28	8	0.15
17j		10	9	ND	28	4	0.35
17k		17	10	ND	51	7	0.31
17l		15	8	ND	44	5	0.25
17m		13	17	ND	58	5	0.29
17n		26	21	ND	51	7	0.36
17o		7	8	ND	84	9	0.201

[a] Each experiment was run in duplicate and the values shown are the average of the two.
[b] ND = not determined.
[c] Racemic mixture.
[d] A mixture of *E* and *Z* isomers.

Subtle differences in kinome selectivity for these analogs were observed, with **17l** exhibiting a slightly better selectivity score. The *cis*-isomer of **17l** (**17o**) was also evaluated, and was found to possess virtually the same in vitro profile as the *trans*-isomer **17l**.

Rat PK was used as a screening model to assess the feasibility of dosing compounds in vivo. The PK profiles of select compounds are shown in Table 5. In comparison to **1**, primary alcohol analog **16a** and secondary alcohol analog **17a** both showed elevated clearance and reduced exposure following intravenous (iv) dosing. Compound **17l** showed clearance similar to the original lead **1**, and improved exposure over both **16a** and **17a**. Good exposure of **17l** In SCID mice was also achieved with an AUC value of 47.48 μM·h following a single 60 mg/kg intraperitoneal (ip) dose.

The antitumor efficacy of **17l** was assessed in a subcutaneous flank-tumor xenograft model in nude mice using the HCT-116 cell line. The compound was dosed intraperitoneally BID using 50% PEG/water as vehicle. Even though substantial dose-dependent tumor growth inhibition was observed (comparable antitumor activity to positive control compound, VX-680), severe body weight loss (>20%) in the high dose group (37.5 mg/kg dosed BID for eight days) complicated the interpretation of the pharmacological effect. A number of factors might have influenced the outcome of the xenograft study, including route of administration, vehicle used



Scheme 4. Reagents and conditions: (a) mCPBA, DCM, rt, 52%; (b) ROH, $B(C_6F_5)_3$, DCM, 90 °C, 5 h; (c) $Pd(OH)_2$, H_2, MeOH, ca. 56% over two steps; (d) **11**, KI, DIEA, DMF, 70 °C, 3 h, 23–62%.

Table 5
Intravenous rat PK profiles for compounds **1**, **9e**, **16a**, **17a**, **17l**[a,b]

Compds	Clearance (mL/min/kg)	Volume of distribution (L/kg)	Half life (h)	AUC (μM·h)
1	28.5	2.15	10.8	2.34
9e	53.3	5.86	8.66	0.757
16a	52.2	9.77	15.4	0.581
17a	45	18.3	11.2	0.644
17l	25.2	2.93	3.36	1.17

[a] 1 mg/kg dosed intravenously.
[b] Sprague Dawley rat, PEG400/water as vehicle.

in the study, dosing schedule, strain of animals, and the possibility of intrinsic toxicity of the lead compounds. Further efforts to improve the overall profiles of the lead compound **17l** will be reported in due course.

In summary, introduction of basic amines to the α-position of the carboxamide terminus of lead compound **1** resulted in analogs with high cellular activity and improved rat PK profiles. Particularly, compound **17l** serves as an excellent lead molecule for further optimization of the overall properties of this series of pan-Aurora kinase inhibitors.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.07.027.

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