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Bioisosteric approach to the discovery of imidazo[1,2-*a*]pyrazines as potent Aurora kinase inhibitors

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ABSTRACT

Our continued effort toward the development of the imidazo[1,2-*a*]pyrazine scaffold as Aurora kinase inhibitors is described. Bioisosteric approach was applied to optimize the 8-position of the core. Several new potent Aurora A/B dual inhibitors, such as **25k** and **25l**, were identified.

Mitosis is a key step in the cell cycle governing the distribution of genetic material to the daughter cells. Errors in this process cause the formation of cells with abnormal chromosome content and lead to genetic instability, a common hallmark of cancer.[1] The Aurora kinase family comprises three highly homologous serine/threonine protein kinases, Aurora A, B, and C. Aurora kinases are critical regulators of cell cycle progression. Aurora A functions in centrosome regulation and mitotic spindle formation; Aurora B is required for correct chromosome alignment, segregation, spindle checkpoint function, and cytokinesis; and although the role of Aurora C is less known, it is believed to be more closely related to Aurora B with overlapping functions and similar localization patterns.[1] Aurora A and B are known to be frequently overexpressed in a wide range of different human tumors, including breast, lung, colon, ovarian, and pancreatic cancers. As enzymes specific for and essential to cell growth and division, inhibitors of Aurora kinase have the potential to be useful for the control of tumor cell growth. Hence, inhibition of Aurora kinases is emerging as an attractive target for cancer treatment. At present, a number of Aurora kinase inhibitors, including Aurora A-selective, Aurora B-selective, and Aurora A and B dual kinase inhibitors are being evaluated in preclinical and clinical assessment for the treatment of cancer.[2] Due to the different role and function of Aurora kinase isoforms in mitosis, currently it is not clear whether compounds with different selectivity profiles within the Aurora family will represent distinct clinical opportunities.

Recently, we reported the discovery of imidazol[1,2-*a*]pyrazines as Aurora kinase inhibitors.[3] Lead compound **1** was identified as Aurora A/B dual inhibitors (IC_{50}: ⩽4 nM and ⩽13 nM, respectively) with submicromolar on-target cell based activity, but with no oral availability (Fig. 1). As we have described earlier,[3] the importance

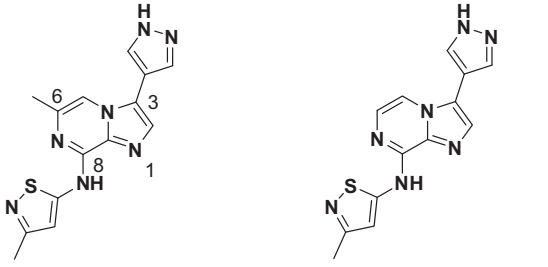

Figure 1. Previously disclosed, potent Aurora kinase inhibitors.

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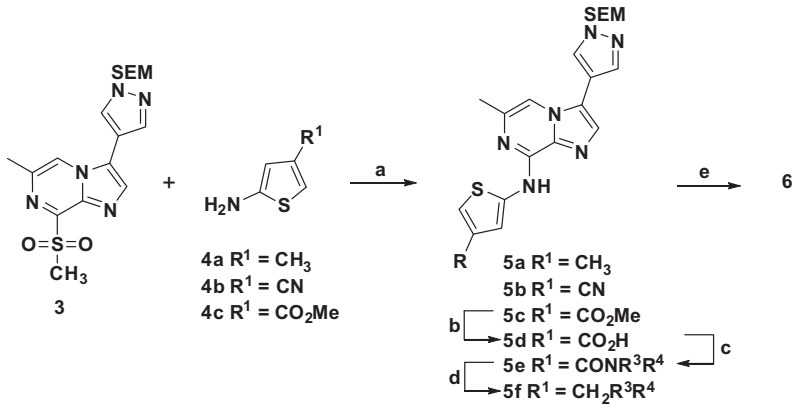
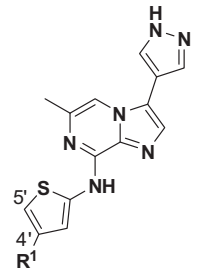


Scheme 1. Reagents and conditions: (a) NaH, DMF, rt; (b) NaOH, THF–MeOH–H_2O, rt; (c) R^3R^4NH, HATU, NMM, DMF, rt; (d) $LiAlH_4$, DCM–Et_2O, 40 °C; (e) 4 N HCl in dioxane, 60 °C.

Table 1
Aurora A and B inhibition data for **6a–h**



Compds	R^1	Aurora A[a] IC_{50} (nM)	Aurora B[a] IC_{50} (nM)	Phos-HH3[a] IC_{50} (nM)
6a	$-CH_3$	30	149	>10,000
6b	$-CN$	74	42	3176
6c	$-CO_2H$	3000	3000	Not tested
6d	$-CO_2CH_3$	1060	2273	Not tested
6e		965	3000	Not tested
6f		3000	3000	Not tested
6g		5	⩽13	>1000
6h		⩽4	⩽13	140

[a] Assay conditions listed in Ref. 6.

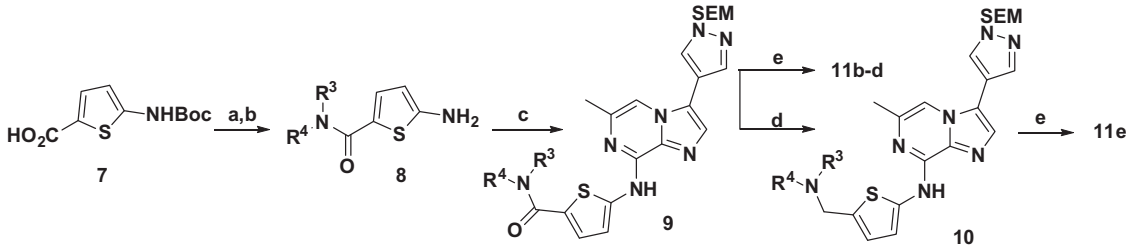
of the sulfur atom of the isothiazole ring for activity, we sought to investigate this further with bioisosteric[4] heterocycles containing a sulfur atom. An additional goal was to optimize for the pharmacokinetic (PK) properties. Keeping the preferred combination of the unsubstituted pyrazole ring at C-3 and the methyl group at C-6, we focused our attention on installing substituted sulfur containing heterocycles such as thiophenes and thiazoles. Herein, we wish to report the synthesis and structure–activity relationships (SAR) of the new thiophene- and thiazole-based Aurora A and B dual inhibitors.

As shown in Scheme 1, a variety of amino thiophene groups (**4a–c**) were appended to the C-8 position of the imidazo-[1,2-*a*]-pyrazine core through the displacement of the methyl sulfone of intermediate **3**[5] to afford compound **5**. Saponification of the methyl ester **5c** resulted in acid **5d** which was then converted to the amides **5e** through HATU-mediated coupling. The corresponding amine **5f** was obtained by reduction of the amide **5e**. Removal of the SEM protecting group under acidic conditions provided final compounds **6a–h**.

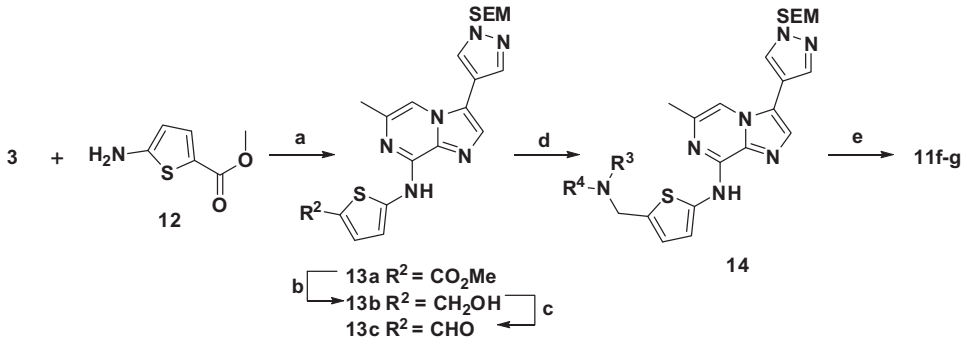
The analogs synthesized were evaluated in the assays[6] and the results are shown in Table 1. The C-4′ position of the thiophene, corresponding to the region originally occupied by the methyl group in isothiazole lead **1**, proved sensitive to changes, as most of the analogs had only moderate or no activity against both Aurora A and B. For example, compound **6a**, the direct analog of **1** but lacking the isothiazole nitrogen, is less potent than **1**. Changing the methyl to a CN group (**6b**) resulted in moderate Aurora A and B activity with weak cell potency. Analogs with substituents such as, carboxylic acid (**6c**), ester (**6d**), and amide (**6e, 6f**), were found to be less active. However, compared to amides (**6e**, **6f**), corresponding amines (**6g**, **6h**) were much more potent for Aurora A and B enzymatic activity including the cellular potency (**6h**).

Unlike the isothiazole, the thiophene has two possible positions (C-4′ and C-5′) that could be substituted to optimize for potency and PK. As shown in Schemes 2–4, we prepared a series of compounds **11a–i** with various substitutents at the C-5′ position of the thiophene ring.

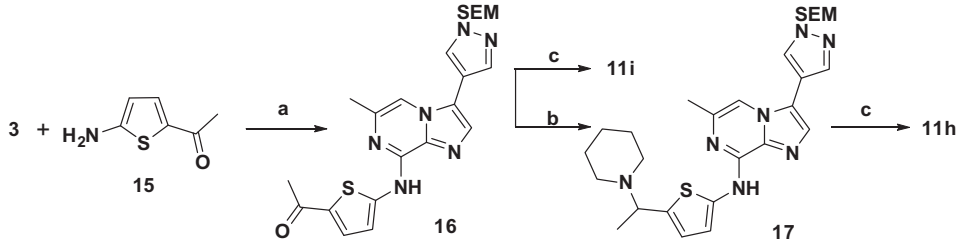
The amide derivatives **11b–d** and the amine analog **11e** were prepared following the sequence presented in Scheme 2. The



Scheme 2. Reagents and conditions: (a) R^3R^4NH, EDCI, Et_3N, DMF, rt; (b) 20% TFA/DCM, rt; (c) **3**, NaH, DMF, rt; (d) $LiAlH_4$, DCM–Et_2O, rt; (e) 4 N HCl in dioxane, rt.

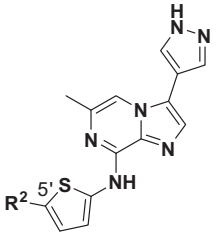


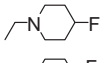
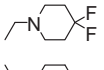
Scheme 3. Reagents and conditions: (a) NaH, DMF, rt; (b) DIBAL, THF, −78 °C; (c) Dess–Martin, DCM, rt; (d) R^3R^4NH, $NaBH(OAc)_3$, DIEA, DCE, rt; (e) 4 N HCl in dioxane, rt.



Scheme 4. Reagents and conditions: (a) NaH, DMF, rt; (b) piperidine, $Ti(OiPr)_4$, rt overnight, then $NaBH_4$, MeOH, rt; (c) 4 N HCl in dioxane, rt.

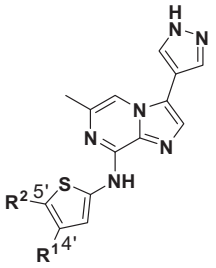
Table 2
Aurora A and B inhibition data for **11a–i**

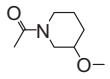
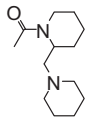


Compds	R^2	Aurora A[a] IC_{50} (nM)	Aurora B[a] IC_{50} (nM)	Phos-HH3[a] IC_{50} (nM)
11a		5	24	1171
11b		⩽4	17	858
11c		⩽4	34	>1000
11d		⩽4	21	>1000
11e		14	13	324
11f		⩽4	⩽13	184
11g		16	23	>1000
11h		99	87	>1000
11i		13	21	>1000

[a] Assay conditions listed in Ref. 6.

Table 3
Aurora A and B inhibition data for **25a–l**



Compds	R^1	R^2	Aurora A[a] IC_{50} (nM)	Aurora B[a] IC_{50} (nM)	Phos-HH3[a] IC_{50} (nM)
11b	–H		⩽4	17	858
25a	$-CH_3$		⩽4	18	278
25b			40	213	2354
25c			⩽4	⩽13	166
25d			83	33	>10,000
25e			⩽4	⩽13	168
25f			2	14	165
25g			⩽4	⩽13	981
25h			⩽4	⩽13	86
25i			⩽4	⩽13	88
25j	$-CH_3$		14	13	324
25k	$-CH_3$		21	⩽13	43
25l			31	12	58

[a] Assay conditions listed in Ref. 6.

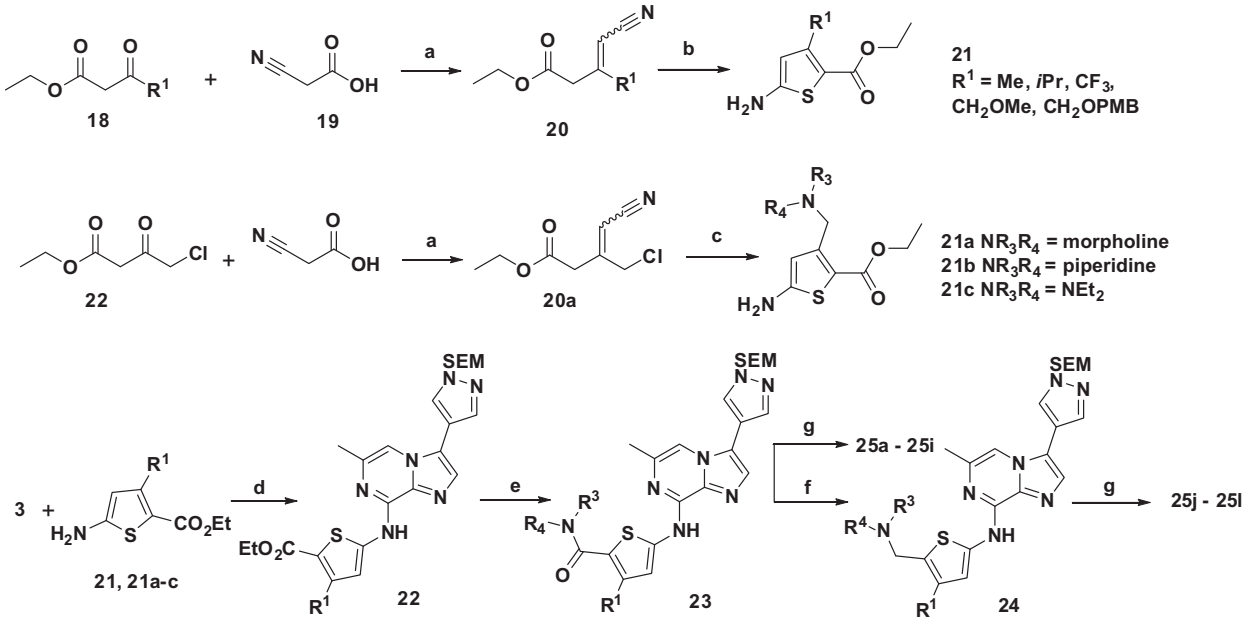
amides, **9**, were obtained from 5-(*tert*-butoxycarbonylamino)thiophene-2-carboxylic acid (**7**) through a three step reaction sequence: amide coupling, Boc deprotection, and sulfone displacement. Lithium aluminum hydride reduction of the amide **9b** provided the amine **10**. Removal of the SEM group from amides **9** and amine **10** afforded the desired analogs **11b–e**.

Cyclic amines, such as the fluoro substituted piperidine analogs **11f** and **11g**, could also be synthesized via the reductive amination route as shown in Scheme 3. The key intermediate aldehyde **13c** was prepared by first transforming methyl ester **13a** to primary alcohol **13b** which was then oxidized using the Dess–Martin reagent.

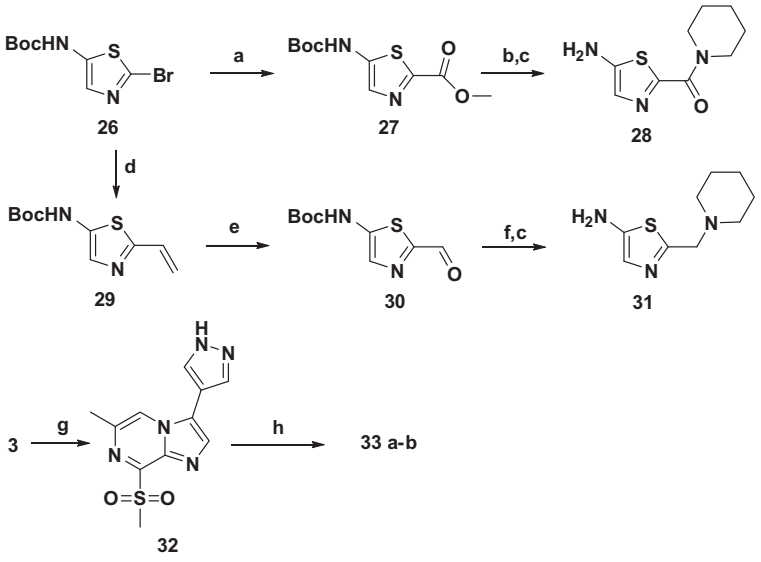
The synthetic route to compound **11i–h** is shown in Scheme 4. Compound **11h** with a methyl group at the benzylic position was prepared through a $Ti(OiPr)_4$-mediated reductive amination reaction of ketone **16** followed by acidic deprotection of the SEM group.

As shown in Table 2, substituents at C-5′ position of the thiophene ring were better tolerated, for example, sulfonamide (**11a**), amide (**11b–d**), amine (**11e–h**), and ketone (**11i**) provided potent Aurora A/B inhibitors with range of modest cellular activities. Converting the amide (**11b**) to the corresponding amine (**11e**) did not provide a significant enhancement in potency which is different from the trend observed in the C-4′ series. In order to minimize the metabolic oxidation of the piperidine ring, fluorine was incorporated. The introduction of one F group at the 4-position of piperidine ring (**11f**) slightly improved the cell potency. In contrast, two fluorine substituents at the 4-position of the piperidine ring (**11g**) resulted in a loss of cell activity. Benzylic site substitution with a methyl group to mitigate oxidation (**11h**) resulted in the loss of Aurora A and B activity.

With the individual C-4′ and C-5′ substituents optimized at the thiophene ring, we directed our next effort toward the preparation



Scheme 5. Reagents and conditions: (a) NH_4OAc, HOAc, PhH, refluxing, Dean-Stark trap; (b) S-flakes, then Et_2NH, EtOH; (c) R^3R^4NH (2 equiv), then S-flakes, EtOH; (d) **3**, NaH, DMF; (e) R^3R^4NH, *i*PrMgCl, THF, 0 °C to rt; (f) $LiAlH_4$, DCM–Et_2O, rt; (g) 4 N HCl in dioxane, rt.



Scheme 6. Reagents and conditions: (a) Pd (dppf)Cl_2, $Mo(CO)_6$, DIEA, EtOH, refluxing; (b) piperidine, $AlMe_3$, THF, −78 °C; (c) TMSI, DCM; (d) tributyl(vinyl)stannane, $Pd(Ph_3)_4$, dioxane, refluxing; (e) $NaIO_4/OsO_4$, dioxane:H_2O (3:1), 2,6-lutidine, rt; (f) piperidine, $NaBH(OAc)_3$, HOAc (cat.), DCM; (g) 2 N HCl in dioxane; (h) **28** or **31**, NaH, DMSO.

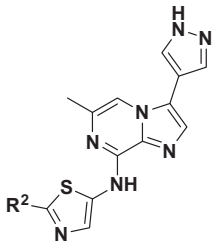
of thiophene derivatives with substituents at both C-4′ and C-5′ position. Scheme 5 outlines the synthesis of C-4′ and C-5′ di-substituted thiophene analogs.

As shown in Scheme 5, amino thiophenes **21** with various substituent at the C-4′ position were synthesized according to the published procedure.[7] A modified route was used to obtain **21a–c** which had different amine group at the C-4′. Ethyl 4-chloro-3-oxobutanoate **22** was condensed with 2-cyanoacetic acid **19** through a Knoevenagel reaction to afford **20a** as a mixture of (*E*)- and (*Z*)-regioisomers. Treatment of **20a** with an excess of different secondary amines followed by sulfur flakes in EtOH provided **21a–c**. Another key reaction was the direct conversion of ethyl ester **22** into the corresponding amide **23** via an *i*PrMgCl mediated reaction.[8]

As shown in Table 3, C-4′ and C-5′ di-substituted thiophene analogs were found to show good activity against both Aurora A and B. A small alkyl group is preferred at C-4′ (R^1 = **H**, **11b** vs R^1 = **Me**, **25a**). However, presumably due to lack of adequate polarity, as the alkyl group becomes larger and branched, the enzymatic and cell potencies began to drop (R^1 = ***i*Pr**, **25b**). Interestingly, R^1 groups at C-4′ with non-acidic polar groups (**25c**, **25e**–**g**) were well tolerated displaying good enzymatic and cell potency. Encouragingly, with more polar R^1 and R^2 groups at the corresponding C-4′ and C-5′ position of thiophene ring led to further improvement in cellular activity was seen (**25h**, **25i**, **25k**, and **25l**), perhaps due to improvement in the physico-chemical properties of these molecules.

In addition to thiophenes as a replacement for the isothiazole, the potential utility of aminothiazoles as bioisosteres was ex-

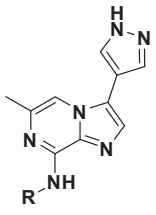
Table 4
Aurora A and B inhibition data for **33a** and **33b**

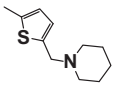
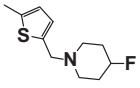
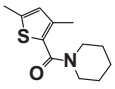
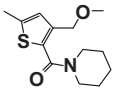
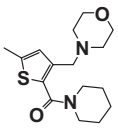


Compds	R^2	Aurora A[a] IC_{50} (nM)	Aurora B[a] IC_{50} (nM)	Phos-HH_3[a] IC_{50} (nM)
33a		178	274	Not tested
33b		⩽4	⩽13	377

[a] Assay conditions listed in Ref. 6.

Table 5
Rat oral PK data of selected compounds



Compds	R	Rat AUC (nM h)[a]
11e		1184
11f		1175
25a		697
25c		212
25e		0

[a] $AUC_{0-6\ h}$ (10 mg/kg, po, in 20% HPBCD).

plored. Key thiazole analogs, **33a** and **33b**, were prepared according to Scheme 6. Better yields were obtained when the amine and amide groups were pre-installed onto the thiazole moiety before the sulfone displacement reaction.

As shown in Table 4, the amide compound **33a** only demonstrated moderate activity against both Aurora A and B, perhaps due to rigid amide group that might misalign favorable interactions. However, more flexible amine **33b** displayed excellent activity for both enzymatic and cell assays.

Several compounds were selected for preliminary pharmacokinetic investigation in rat[9] and the results are reported in Table 5. While low exposure was observed for all three amide analogs (**25a**, **25c**, and **25e**), amine derivatives showed better oral PK in the rat (**11e** and **11f**). Combined with their good enzymatic and cell potency, the amine containing thiophene compounds such as **11e** and **11f** provide a good template for further development of orally bioavailable Aurora kinase inhibitors.

In summary, replacement of the isothiazole in the lead compound **1** by a bioisoteric moiety, such as thiophene and thiazole, led to potent Aurora A/B inhibitors that displayed good cell based activity. SAR development in the thiophene and thiazole series will provide the basis for development of more potent and orally bioavailable Aurora kinase inhibitors.[10,11]

Acknowledgment

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6. *Biochemical assays*: Aurora A and Aurora B kinase assays were performed in low protein binding 384-well plates. Compounds were diluted in 100% DMSO to the desired concentrations. For the Aurora A assay, each reaction consisted of 8 nM enzyme (Aurora A, Upstate), 100 nM Tamra-PKAtide (Molecular Devices, 5TAMRA-GRTGRRNSICOOH), 25 μM ATP, 1 mM DTT, and kinase buffer (10 mM Tris, 10 mM $MgCl_2$, 0.01% Tween 20). For the Aurora B assay, each reaction consisted of 26 nM enzyme (Aurora B, Invitrogen), 100 nM Tamra-PKAtide (Molecular Devices, 5TAMRA-GRTGRRNSICOOH), 50 μM ATP, 1 mM DTT, and kinase buffer (10 mM Tris, 10 mM $MgCl_2$, 0.01% Tween 20). Dose–response curves were plotted from inhibition data generated in duplicate, from 8 point serial dilutions of inhibitory compounds. Concentration of compound was plotted against kinase activity, calculated by degree of fluorescent polarization. To generate IC_{50} values, the dose–response curves were then fitted to a standard sigmoidal curve and IC_{50} values were derived by non-linear regression analysis. *Immunofluorescent assays*: HCT-116 cells were plated at 15,000 cells per well in poly-D-lysine coated black micro-clear 384-well tissue culture plates. For the phos-Histone H3 assay, cells were first treated with 0.4 mg/ml nocodazole. Sixteen hours later cells were treated in triplicate with compound (0.1% final DMSO concentration) for 1 h. Cells were fixed with Prefer® fixation solution (Anatech) plus 1000 nM Hoechst 33342 dye and incubated for 30 min at room temperature. The fixation solution was removed and cells were washed with PBS. Cells were permeabilized with 0.2% Triton-X in PBS and incubated for 10 min. Cells were washed with PBS and incubated with PBS containing 3% FBS for 30 min. Cells were then stained overnight at 4 °C with Phos-Histone H3 (Ser10)-Alexa Flur 488 Conjugate antibody (Cell Signaling) solution in PBS plus 3% FBS. Cells were washed with PBS and then immunofluorescence images were captured at 10× with BD Pathway 855 automated fluorescent microscope (BD Bioscience). Percent positive cells were quantitated using Hoechst staining for cell number using Attovision software (BD Bioscience). To generate IC_{50} values, the dose–response curves were then fitted to a standard sigmoidal curve and IC_{50} values were derived by non-linear regression analysis.
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