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7-(Pyrazol-4-yl)-3*H*-imidazo[4,5-*b*]pyridine-based derivatives for kinase inhibition: Co-crystallisation studies with Aurora-A reveal distinct differences in the orientation of the pyrazole *N*1-substituent

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ABSTRACT

Introduction of a 1-benzyl-1*H*-pyrazol-4-yl moiety at C7 of the imidazo[4,5-*b*]pyridine scaffold provided **7a** which inhibited a range of kinases including Aurora-A. Modification of the benzyl group in **7a**, and subsequent co-crystallisation of the resulting analogues with Aurora-A indicated distinct differences in binding mode dependent upon the pyrazole *N*-substituent. Compounds **7a** and **14d** interact with the P-loop whereas **14a** and **14b** engage with Thr217 in the post-hinge region. These crystallographic insights provide options for the design of compounds interacting with the DFG motif or with Thr217.

Over the last decade, extensive research has been directed towards the discovery of small molecule inhibitors of the Aurora protein kinases as anticancer agents. This effort led to the identification of multiple structurally diverse chemotypes for Aurora kinase modulation.[1–5] In addition to small molecules inhibiting all three Aurora isoforms A, B and C, several compounds displaying Aurora isoform selectivity have also been reported including AZD1152 which selectively inhibits Aurora-B,[6] and the selective Aurora-A inhibitor MLN8237.[7] Several of these small-molecule inhibitors (e.g., PHA-739358,[8,9] AZD1152 and MLN8237) have progressed through preclinical development into clinical evaluation for the treatment of a range of human malignancies.[1–5]

We have previously reported imidazo[4,5-*b*]pyridine-based inhibitors of Aurora kinases including **1** (CCT137690),[10] the dual FLT3/Aurora kinase inhibitor **2** (CCT241736),[11] and compound **3** which selectively inhibits Aurora-A over Aurora-B[12] (Fig. 1). In our work related to the discovery of **3**, modification of the imidazo[4,5-*b*]pyridine scaffold at C7 included the introduction of a 1-benzyl-1*H*-pyrazol-4-yl moiety that provided entry into 7-(pyrazol-4-yl)-3*H*-imidazo[4,5-*b*]pyridine-based derivatives. Herein, we report our medicinal chemistry effort aimed at improving the pharmacological profile for this class of compounds, and present kinome profiling data that indicate promiscuous kinase inhibition for this subseries. In addition, we report ligand/Aurora-A protein crystallographic data that show different orientations for the substituent on the pyrazole ring suggesting that the 7-(pyrazol-4-yl)-3*H*-imidazo[4,5-*b*]pyridine scaffold could be utilised for the design of compounds that additionally interact with the DFG motif or with Thr217, the latter tactic has been demonstrated to enhance Aurora-A over Aurora-B selectivity.[12]

Synthesis of 7-substituted imidazo[4,5-*b*]pyridine derivatives **7a**–**e** (Table 1) is shown in Scheme 1. Key intermediates **5a**–**e** were obtained via Suzuki reaction between 4-chloro-3-nitropyridin-2-amine (**4**) and the requisite boronic acid or boronic acid pinacol ester coupling partner. 1-(3,4-Difluorobenzyl)-, 1-(4-fluorobenzyl)-, and 1-(4-chlorobenzyl)-1*H*-pyrazole-4-boronic acid pinacol esters were prepared by heating 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole (**11**) with the appropriate benzyl

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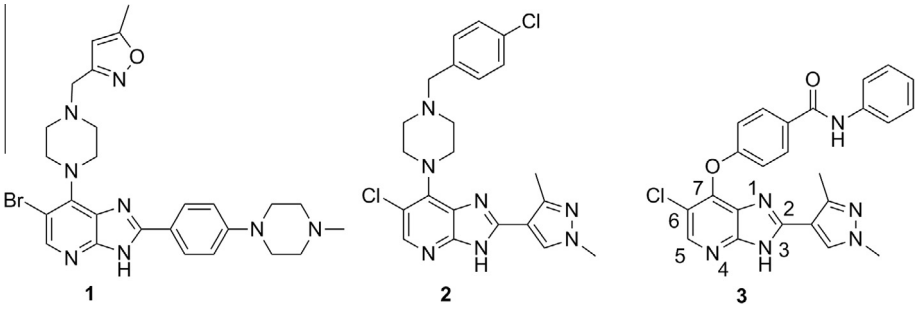
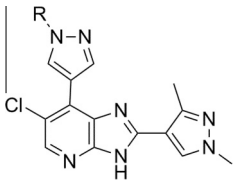
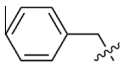
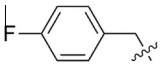
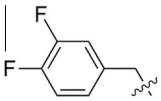
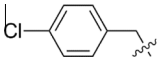
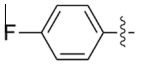


Figure 1. Imidazo[4,5-*b*]pyridine-based inhibitors of Aurora kinases.

Table 1
C7-Pyrazole modifications



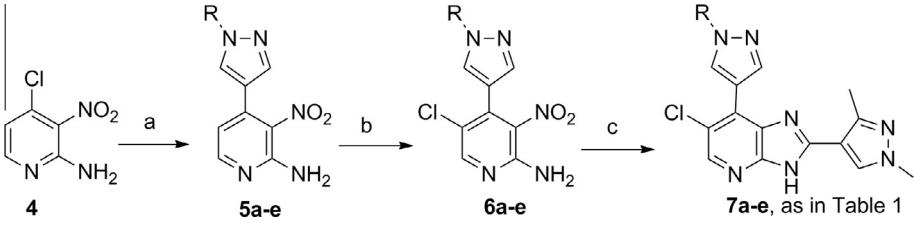
Compd	R	Aurora-A IC_{50} (μM)	Aurora-B IC_{50} (μM)	MLM/HLM% met/30 min
7a		0.212 ± 0.107	0.461 ± 0.147	40/43
7b		0.182	0.347	52/39
7c		0.322	0.458	31/28
7d		0.190	0.271	37/16
7e		>10	>10	31/20

IC_{50}s are mean values of two independent determinations or mean (±SD) for $n > 2$.[17]
MLM/HLM: percentage of compound metabolised after a 30 min incubation.[18]

bromide at 80 °C for 2–3 h in acetonitrile in the presence of Cs_2CO_3, reaction conditions previously reported for the synthesis of 1-benzyl-3-heterocyclic pyrazoles.[13] (1-Benzyl-1*H*-pyrazol-4-yl)boronic acid and (1-(4-fluorophenyl)-1*H*-pyrazol-4-yl)boronic acid, required for the synthesis of **5a** and **5e** respectively, were commercially available. *N*-chlorosuccinimide-mediated C5-pyridine chlorination was followed by reaction with 1,3-dimethyl-1*H*-pyrazole-4-carbaldehyde in the presence of $Na_2S_2O_4$, as previously described,[11,12,14] to afford the imidazo[4,5-*b*]pyridine derivatives **7a**–**e** (Scheme 1, Table 1).

The 2-amino-3-nitro-pyridine derivative **9** (Scheme 2), key intermediate for the synthesis of **14a** and **14b** (Table 2), was obtained from 4-chloro-3-nitropyridin-2-amine (**4**) by a Suzuki cross-coupling reaction to (1-(3-(methoxycarbonyl)benzyl)-1*H*-pyrazol-4-yl)boronic acid pinacol ester, prepared by reacting **11** with methyl 3-(bromomethyl)benzoate[13], followed by C5-pyridine chlorination with *N*-chlorosuccinimide (Scheme 2). An attempt to prepare **10a** directly from the methyl ester intermediate **9** upon treatment with dimethylamine under microwave irradiation resulted in formation of the corresponding carboxylic acid. Coupling of this acid with dimethylamine via HATU carboxyl activation provided **10a** (Scheme 2). Starting from the methyl ester intermediate **9**, access to **10b** was achieved by alkaline ester hydrolysis followed by HATU-mediated coupling with 1-methylpiperazine (Scheme 2). Imidazo[4,5-*b*]pyridine ring formation to afford **14a** and **14b** (Table 2) was effected by reacting **10a** and **10b** with 1,3-dimethyl-1*H*-pyrazole-4-carbaldehyde in the presence of $Na_2S_2O_4$ as previously described.[11,12,14] The *ortho*-substituted derivative **14c** (Table 2) was prepared by a procedure analogous to that described for its *meta*-isomer **14b** (Scheme 2, Table 2).

Access to 2-amino-3-nitro-pyridine derivative **13** (Scheme 3), key intermediate for the preparation of **14e**–**g**, was achieved by alkylation of 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole (**11**) with 3-(bromomethyl)benzaldehyde[13] followed by a Suzuki cross-coupling reaction with 4-chloro-3-nitropyridin-2-amine (**4**) (Scheme 3). Reductive amination of the benzaldehyde moiety in **13** was accomplished under standard conditions ($NaBH(OAc)_3$/AcOH with methylamine or pyrrolidine, and $NaBH_3CN$ with dimethylamine). The synthesis of **14e**–**g** (Table 2) was finalised by the C5-pyridine chlorination of the requisite intermediate followed by imidazo[4,5-*b*]pyridine ring formation as described for the preparation of **7a**–**e** (Scheme 1). Finally, the isoxazole derivative **14d** (Table 2) was prepared from **4** by a route analogous to that described for the synthesis of **7a**–**e** (Scheme 1). For this synthetic sequence, (1-((5-methylisoxazol-3-yl)methyl)-1*H*-pyrazol-4-yl) boronic acid pinacol ester, required for the Suzuki cross-coupling reaction, was prepared by alkylation of 4-(4,4,5,5-tetramethyl-



Scheme 1. Reagents and conditions: (a) boronic acid or boronic acid pinacol ester, THF/H_2O, $Pd(dppf)Cl_2$, Na_2CO_3, 80 °C, 3.5–26 h or DME, $Pd(dppf)Cl_2$, 1 M aqueous Na_2CO_3, 150 °C, microwave irradiation, 15 min (for the introduction of 1-(4-fluorophenyl)-1*H*-pyrazol-4-yl); (b) *N*-chlorosuccinimide, CH_3CN, reflux, 3.5–7 h; (c) EtOH, 1,3-dimethyl-1*H*-pyrazole-4-carbaldehyde, 1 M aqueous $Na_2S_2O_4$, 80 °C, 16–20 h.