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Design and evaluation of 3,6-di(hetero)aryl imidazo[1,2-*a*]pyrazines as inhibitors of checkpoint and other kinases

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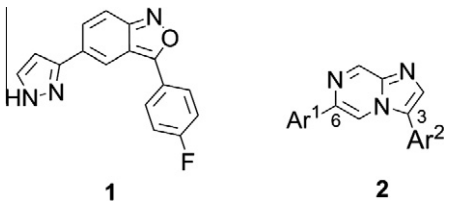
ABSTRACT

A range of 3,6-di(hetero)arylimidazo[1,2-*a*]pyrazine ATP-competitive inhibitors of CHK1 were developed by scaffold hopping from a weakly active screening hit. Efficient synthetic routes for parallel synthesis were developed to prepare analogues with improved potency and ligand efficiency against CHK1. Kinase profiling showed that the imidazo[1,2-*a*]pyrazines could inhibit other kinases, including CHK2 and ABL, with equivalent or better potency depending on the pendant substitution. These 3,6-di(hetero)aryl imidazo[1,2-*a*]pyrazines appear to represent a general kinase inhibitor scaffold.

Protein kinase enzymes play vital roles in the control of cell cycle progression, apoptosis, growth and metabolism and are well established as potential targets for intervention in a number of diseases including cancer.[1] There is a need to identify new chemical classes of kinase inhibitors, including novel heterocycles that fulfil the pharmacophore requirements for occupying the ATP-binding site of the enzymes.[1,2] Checkpoint kinase 1 (CHK1) is an oncology target of current interest as CHK1 inhibitors have the potential to enhance the efficacy of cytotoxic treatments.[3–5] CHK1 is a serine/threonine kinase that is phosphorylated and activated in response to DNA damage, initiating a signalling cascade culminating in cell cycle arrest in the S or G2/M phases. Inhibition of CHK1 has been shown to abrogate these checkpoints leading to enhanced tumour cell death following DNA damage by a range of chemotherapeutics. Tumour cells deficient in the tumour suppressor p53 and lacking intact G1 checkpoints are particularly dependent on S and G2/M checkpoints, and are therefore expected to be sensitised to genotoxic treatment in the presence of a CHK1 inhibitor. Several chemotypes of CHK1 inhibitor have been described, including compounds in early clinical trials.[3–6]

We have previously reported a screening strategy that identified several low molecular weight compounds, or templates, as ATP-competitive CHK1 inhibitors, including the weakly active benzisoxazole (Fig. 1, **1**) (26% inhibition at 250 μM).[7] We have investigated the progression of a number of these hits to identify chemical series with good prospects for optimisation into potent and selective leads. We describe here new imidazo[1,2-*a*]pyrazines (Fig. 1, **2**) and the development of an efficient synthetic procedure to make analogues of this scaffold. Improvements in CHK1 potency were achieved, while kinome screening indicated the new compounds to have wider applicability as a kinase inhibitor chemotype.

A literature search indicated that benzisoxazole kinase inhibitors were already documented.[8,9] Indazoles with strikingly similar substitution patterns have been reported as JNK inhibitors.[10,11] The design of an alternative 6,5-bicyclic template that would provide increased affinity for CHK1 was therefore pursued and a novel 3,6-disubstituted imidazo[1,2-*a*]pyrazine core **2** was envisaged. Although imidazo[1,2-a]pyrazines bearing heteroatom 3- and 6-substituents have been reported as kinase inhibitors[12] there are



1 **2**

Figure 1. Benzisoxazole **1** and the imidazo[1,2-*a*]pyrazine core **2**.

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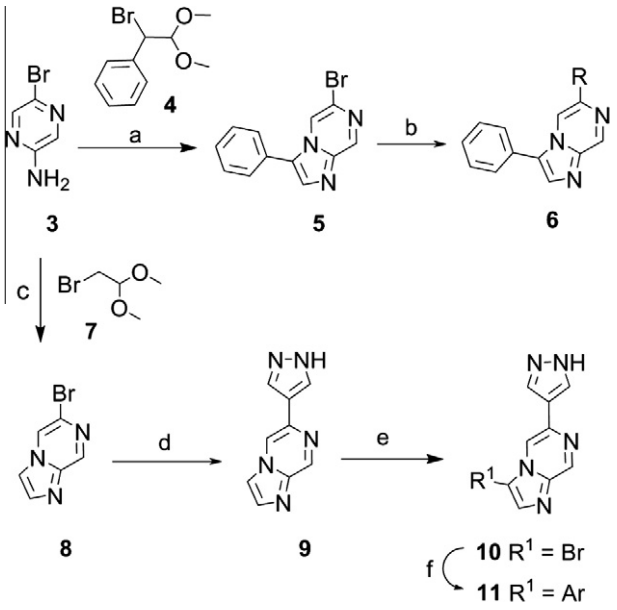
only limited examples of similar 3,6-di(hetero)aryl imidazo[1,2-*a*]pyrazines in the patent literature.[13,14]

A small array of 3-phenyl imidazo[1,2-*a*]pyrazines were synthesised which displayed encouraging CHK1 activity (Table 1). α-Bromophenylacetaldehyde dimethyl acetal **4** was deprotected with HBr followed by condensation of the resulting aldehyde with 2-amino-5-bromopyrazine **3** to afford 6-bromo-3-phenyl imidazopyrazine **5** (Scheme 1).[15] Suzuki–Miyaura coupling (or in the case of **18** a Stille coupling) installed the 6-substituent. Of the nine examples synthesised, the 4-pyrazolyl and 4-pyridyl analogues **13** and **20** were the most potent against CHK1. The pyrrole **16**, indole **17** and 3-pyrazole analogue **12**, which most closely mirrors the substitution in the original hit **1**, were less active. N-Substitution of the 4-pyrazole (**14**, **15**) led to a reduction in potency as did replacing the 4-pyridyl with the 2- or 3- regioisomers (**18**, **19**).

The binding mode of **13** in CHK1 was determined by X-ray crystallography, through soaking of the ligand into crystals of apo-CHK1[7] (PDB code 2XF0, Fig. 2, Panel A). This showed a hydrogen bond from N1 to the amide NH of Cys87 with the suggestion of a second favourable contact with the hinge region in the form of a $C_{(Ar)}H\ldots O$ interaction between C2 and the carbonyl of Cys87. The pyrazole ring pointed towards the interior pocket of the enzyme which has been targeted to achieve selectivity for CHK1.[16] The phenyl 3-substituent was directed towards the ribose pocket and solvent.

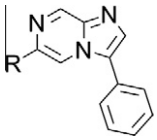
The ligand efficiencies (L.E.)[17] for these first analogues, calculated using the compact formula L.E. = $[-1.4 \times \log_{10}(IC_{50}\ (M))]$/(number of heavy atoms)[17], were modest (Table 1). As the 3-substituent of the imidazo[1,2-*a*]pyrazine followed a vector towards the polar environment of the ribose pocket and solvent exposed region, further analogues were synthesised based on the most active 6-(4-pyrazolyl) analogue **13** but with more hydrophilic 3-substituents. As shown in Scheme 1, the synthesis allowed the point of diversity on the scaffold to be elaborated last. After treatment with acid, bromoacetaldehyde dimethyl acetal **7** was condensed with 2-amino-5-bromopyrazine **3** to form the 6-bromo-imidazole bicycle **8**. Palladium-mediated coupling with pyrazole-4-boronic acid pinacol ester installed the preferred substituent in the 6-position (**9**), before bromination gave the advanced intermediate **10** for the final Suzuki–Miyaura reactions.

A number of the compounds showed increased potency against CHK1 (Table 2), notably the bis-pyrazolyl analogues **22** and **24**. While still inhibiting in the micromolar range, the ligand efficiencies of **22** and **24** were significantly improved. With the exception of the highly lipophilic benzyl ether **25** and the 3-pyridyl analogue **21**, the other compounds displayed weak CHK1 activity. X-ray crystallography showed **24** bound in an identical manner to **13** (PDB code 2XEZ, Fig. 2, Panel B), with the H-bond from N1 and



Scheme 1. Reagents and conditions: (a) (i) **4**, 48% HBr/H_2O, 105 °C, 10 min, μwave; (ii) $NaHCO_3$, IPA, then **3**, 150 °C, 20 min, μwave; 44% yield; (b) $RB(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3, MeCN/H_2O, 150 °C, 20 min μwave; 35–94%; (c) (i) **7**, 48% HBr/H_2O, 100 °C, 2 h; (ii) $NaHCO_3$, IPA, then **3**, 100 °C, 3 h, 76%; (d) 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole, $Pd(PPh_3)_4$, Na_2CO_3, MeCN/H_2O, 150 °C, 20 min μwave, 73%; (e) NBS, DMF, rt, 2.5 h, 37%; (f) $R^1B(OH)_2$, $Pd(PPh_3)_4$, 0.5 M Na_2CO_3, DMF, 150 °C, 30 min. 7–20%.

Table 1
CHK1 activity of 3-phenylimidazo[1,2-*a*]pyrazines



No.	R	CHK1 IC_{50}[a] (μM)	L.E.[b]
12	1*H*-Pyrazol-3-yl	>100	n.d.[d]
13	1*H*-Pyrazol-4-yl	30[c] (28, 31)	0.32
14	1-Methylpyrazol-4-yl	72	0.28
15	1-Benzylpyrazol-4-yl	208	0.19
16	1*H*-Pyrrol-2-yl	>100	n.d.
17	1*H*-Indol-3-yl	>100	n.d.
18	2-Pyridyl	>100	n.d.
19	3-Pyridyl	139	0.26
20	4-Pyridyl	52	0.29

[a] Single determination in DELFIA assay.[7] Standard inhibitor staurosporine gave IC_{50} = 2.1 (±1.8) nM.
[b] Ligand efficiency (kcal mol^{-1} non-H $atom^{-1}$.
[c] Mean of two independent determinations, individual values in parentheses.
[d] Not determined.

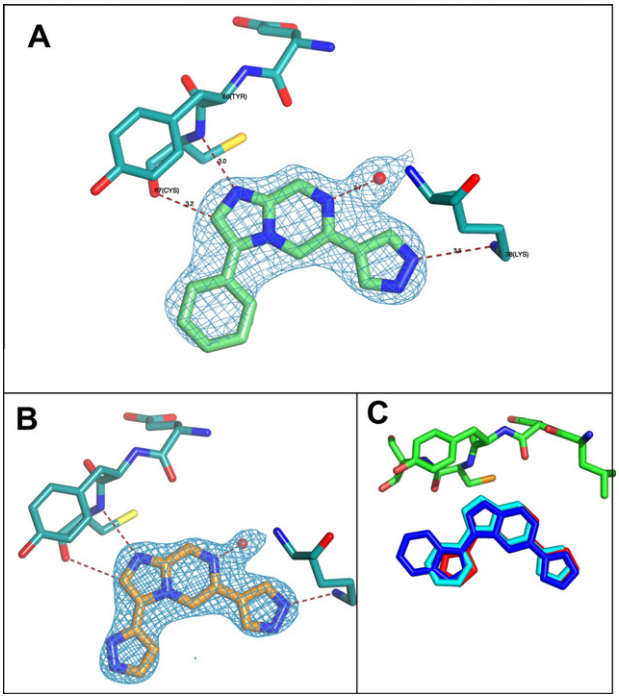
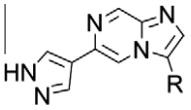
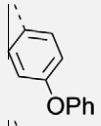
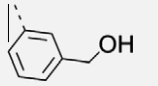
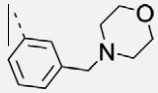
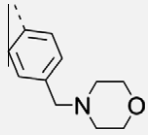


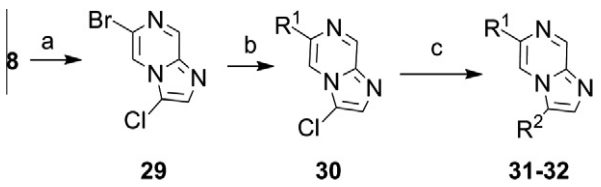
Figure 2. (A) Crystal structure of hit **13** bound to the CHK1active site. $2F_o - F_c$ map contoured at 1.0σ represented by blue mesh (PDB code 2XF0); (B) Crystal structure of hit **24** bound to the CHK1active site. $2F_o - F_c$ map contoured at 1.0σ represented by blue mesh (PDB code 2XEZ); (C) Overlay of **13**, **24** and **31g** relative to the hinge region of CHK1, cyan = **13**, red = **24**, blue = **31g** (PDB code 2XEY), green = CHK1.

Table 2
CHK1 activity of 6-(1*H*-pyrazol-4-yl)imidazo[1,2-*a*]pyrazines



No.	R	CHK1 IC_{50}[a] (μM)	L.E.[b]
21	3-Pyridyl	>100[c]	n.d.[d]
22	1*H*-Pyrazol-4-yl	5.0 (2.2, 7.7)	0.39
23	1-Methylpyrazol-4-yl	22 (21, 23)	0.33
24	1*H*-Pyrazol-3-yl	1.5 (1.4, 1.6)	0.43
25		>100[c]	n.d.
26		46 (34, 58)	0.28
27		23[c]	0.24
28		22 (15, 31)	0.24

[a] Mean of two independent determinations unless otherwise stated, individual values in parentheses. Standard inhibitor staurosporine gave IC_{50} = 2.1 (±1.8) nM.
[b] Ligand efficiency (kcal mol^{-1} non-H $atom^{-1}$).
[c] Single determination.
[d] Not determined.

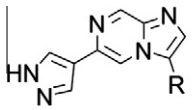


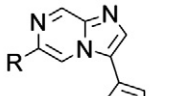
Scheme 2. Reagents and conditions: (a) NCS, DCM, reflux, 5.5 h, 96% yield; (b) $R^1B(OH)_2$, $Pd(PPh_3)_4$, 0.5 M Na_2CO_3, MeCN, 120 °C, 1 h, μwave, 42–75%; (c) $R^2B(OH)_2$, $Pd(PPh_3)_4$, 0.5 M Na_2CO_3, DMF, 150 °C, 20 min, μwave, 26–31%.

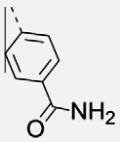
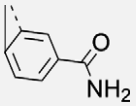
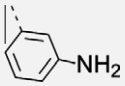
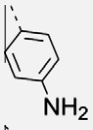
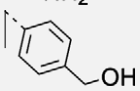
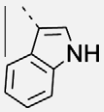
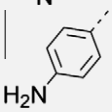
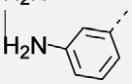
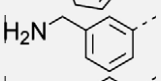
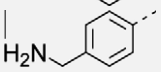
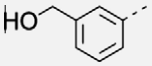
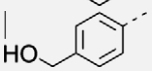
$C_{(Ar)}H...O$ interaction from C2 to Cys87. A better interaction of the nitrogen on the pendant 6-(1*H*-pyrazol-4-yl) group with Lys38 was seen for **24** compared to **13** (2.7 Å vs 3.5 Å). The 3-(1*H*-pyrazol-3-yl) substituent of **24** did not make any additional H-bonds to the protein, with no atoms on either side within reasonable H-bonding distances or angles of the ligand. The increase in potency of **24** over the phenyl-substituted **13** may therefore be due to the improved interaction with Lys38. The 3-pyrazole ring was modelled with the nitrogen atoms pointing out towards solvent though the data are not of sufficiently high resolution to discriminate this from the alternative orientation with the pyrazole rotated by 180°.

To enable elaboration of the bicyclic scaffold at both the 3- and 6-positions, a sequential coupling strategy was sought. Thus chlorination of **8** gave the 3-chloro-6-bromoimidazo[1,2-*a*]pyrazine **29** (Scheme 2) and Suzuki–Miyaura coupling of phenylboronic acid was attempted under various conditions. Most of these led to mixtures of the desired 3-chloro-6-phenyl product and the related dechloro byproduct. However, specific conditions were identified ($Pd(PPh_3)_4$, Na_2CO_3, MeCN/water, 120 °C, 60 min, μwave) that gave both excellent selectivity for reaction at the 6-position and left the 3-chloro group in place. Using the same base and catalyst combination but changing the solvent to DMF and raising the

Table 3
CHK1 activity of selected 3,6-disubstituted imidazo[1,2-*a*]pyrazines prepared by parallel synthesis


31a-g


32a-i

Compd	R	Chk1 IC_{50}[a] (μM)
31a		>100[b]
31b		>100[b]
31c		>100[b]
31d		26 (12, 40)
31e		96[b]
31f		43 (34, 52)
31g		6.5 (5.9,7.1)
32a		25 (16, 34)
32b		17 (14, 21)
32c		15 (12, 18)
32d		15 (14, 17)
32e		28 (19, 37)
32f		3.5 (3.2, 3.7)
32g		2.0 (1.6, 2.3)
32h		29 (19, 39)
32i		39 (21, 57)

[a] Mean of two independent determinations unless otherwise stated, individual values in parentheses. Standard inhibitor staurosporine gave IC_{50} = 2.1 (±1.8) nM.
[b] Single determination.

Table 4
Kinase profiling for selected compounds by mobility shift assay and IC_{50} determinations

Compound	Kinase inhibitory activities[a]																
	AurA	RSK1	Erk1	PKD2	CK1d	CHK1	CHK1 IC_{50}[b] (μM)	ABL1	ABL1 IC_{50}[c] (μM)	FYN	LYN	CHK2	CHK2 IC_{50}[b] (μM)	LCK	SRC	GSK3β	GSK3β IC_{50}[c] (μM)
12	41	11	4	−14	61	4	>100[d]	42	–	68	53	10	–	52	43	22	–
13	47	37	7	8	73	16	30	61	–	58	57	50	6	53	43	54	–
20	21	14	6	−6	44	8	52[d]	31	–	45	46	18	–	28	35	80	3.1
22	86	31	5	11	69	40	5	90	0.44	97	94	67	6.8	86	75	31	–
23	62	45	8	1	48	25	22	50	–	33	24	57	15	63	46	53	–
24	95	72	10	26	69	75	1.5	63	–	88	74	95	0.48	86	85	58	–
26	45	39	10	0	60	12	46	53	–	36	37	34	–	35	39	57	–
31g	87	71	65	55	73	68	6.5	73	–	62	59	82	1.3	66	74	87	5.9
32g	73	64	3	53	55	62	2	95	0.24	100	99	95	0.12	100	97	17	–
32h	45	30	5	14	44	32	29	96	–	99	99	79	1.7	99	97	57	–
32i	89	48	6	10	52	38	39	95	0.30	99	98	57	2.2	96	94	44	–
H89[e]	75	91	48	85	53	90	–	11	–	13	28	81	–	52	21	11	–
(±SEM)	±1.8	±0.9	±1.7	±1.2	±1.2	±0.8	–	±1.3	–	±0.8	±1.1	±0.8	–	±1.9	±1.4	±1.1	

[a] % Inhibition of kinases in a mobility shift assay[21] at 10 μM concentration of test compound and [ATP] = $K_{m,ATP}$ for each individual kinase. Single point determination. Orange >80% inhibition, yellow = 50–80% inhibition.
[b] IC_{50} determined in DELFIA assay.[7] Mean of two independent determinations unless otherwise stated.
[c] IC_{50} determined in Z'-Lyte assay.[23]
[d] Single determination.
[e] Mean (±SEM)% inhibition for internal standard H89, a pan kinase inhibitor,[24] n = 15 determinations.

reaction temperature to 150 °C allowed the 3-chloro substituent to be subsequently derivatised. By carrying out the two reactions in tandem and using automated HPLC purification for the final compounds, a larger number of compounds were prepared (Table 3). Compound **31g** was shown by crystallography to bind identically to the previous examples (Fig. 2C).

Despite a variety of functionality introduced onto the 3- and 6-substituents, the activity for these compounds at CHK1 was not improved over that of **24**. In addition, counterscreening against the structurally distinct enzyme CHK2, also a potential oncology target,[18] indicated little selectivity. For example, compounds **24**, **31g** and **32g** gave CHK2 IC_{50} values[7] of 0.48 μM, 1.3 μM and 0.12 μM, respectively (Table 4). Further selectivity profiling revealed other significant kinase inhibitory activities associated with the scaffold.

A selection of 11 analogues were tested at 10 μM inhibitor concentration against a panel of 24 kinases using a mobility shift assay[20] (Table 4, [ATP] = $K_{m,ATP}$ for each kinase,[21,22] see Supplementary data). At this concentration none of the selected imidazo[1,2-*a*]pyrazines showed greater than 50% inhibition against MAPKAPK2, PKC, PRAK, MET, ERK2, PKA, AKT2, INSR, p38a, AKT1 and MSK1. However activity was observed against the remaining 13 kinases by one or more of the compounds. A group of structurally similar analogues (**32g**, **32h**, **32i**) strongly inhibited (>94% @ 10 μM) the tyrosine kinases ABL, FYN, LYN, LCK and SRC. Compounds **22**, **24**, **31g** and again **32i** were inhibitors of AurA. To validate the screening results, IC_{50} determinations were made for the most potent inhibitors against CHK2, ABL1 and GSK3β,[23] and good agreement was seen between these data and the single-point inhibitions. Notably, sub-micromolar potency was observed for inhibition of ABL1 and CHK2 with these compounds.

The elaboration of other template screen hits, which were evaluated in parallel with **1**, led to potent and selective CHK1 inhibitors which were able to bind in the interior pocket of the enzyme.[19] In view of this, and given the low CHK1 potency and selectivity seen with the compounds described here, the imidazo[1,2-*a*]pyrazines were not progressed further against CHK1. Our experience shows how scaffold morphing from fragment-like hits can generate new inhibitor scaffolds, but also highlights the benefit of monitoring kinase selectivity during the development of the new scaffolds. We speculate that the single explicit hydrogen bond from the core imidazo[1,2-*a*]pyrazine to the hinge region may result in an increased importance of the interactions formed by the two pendant aryl groups to determine affinity and selectivity, and may also result in changes in orientation of the core between kinase targets. It may however be possible to modulate the selectivity profile of the imidazo[1,2-*a*]pyrazines by specific choice of the 3- and 6-substituents.

In summary, scaffold hopping from a very weak benzisoxazole hit generated novel 3,6-di(hetero)aryl imidazo[1,2-*a*]pyrazine ATP-competitive inhibitors of CHK1. By developing synthetic routes that allowed late stage derivatisation of the scaffold, we quickly synthesised analogues with improved potency and ligand efficiency, leading to micromolar inhibitors of CHK1. Kinase profiling showed that several of the imidazo[1,2-*a*]pyrazines inhibited other kinases with equivalent or better potency. The selectivity profile of the compounds varied with the nature of the pendant substituents. The 3,6-di(hetero)aryl imidazo[1,2-*a*]pyrazine scaffold may therefore be generally useful for the development of new 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.2010.05.096.

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