Article

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Fragment-based discovery of pyrazolopyridones as JAK1 inhibitors with excellent subtype selectivity

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ABSTRACT: Herein we report the discovery of a series of JAK1-selective kinase inhibitors with high potency and excellent JAK family subtype selectivity. A fragment screening hit **1** with a pyrazolopyridone core and a JAK1 bias was selected as the starting point for our fragment-based lead generation efforts. A two-stage strategy was chosen with the dual aims of improving potency and JAK1 selectivity: Optimization of the lipophilic ribose pocket-targeting substituent was followed by introduction of a variety of P-loop-targeting functional groups. Combining the best moieties from both stages of the optimization afforded compound **40**, which showed excellent potency and selectivity. Metabolism studies *in vitro* and *in vivo* together with an *in vitro* safety evaluation suggest that **40** may be a viable lead compound for the development of highly subtype-selective JAK1 inhibitors.

INTRODUCTION

The Janus kinase (JAK) family of tyrosine kinases plays a central role in the cytokine-dependent regulation of proliferation, survival, differentiation and function of diverse cells. It consists of four closely related proteins (JAK1, JAK2, JAK3 and TYK2) that transduce signaling from cytokine receptors by phosphorylation and subsequent activation of signal transducers and activators of transcription (STATs).[1,2] JAKs are critically involved in immune response in health and disease.[1,3] The understanding of the role of cytokines in the pathophysiology of immune-mediated diseases and the identification of the critical role of JAKs in cytokine signaling prompted the idea that inhibiting the activity of JAKs might provide a new immunomodulatory therapeutic approach. Tofacitinib, a first-generation JAK inhibitor targeting the activity of JAK1, JAK2 and JAK3, was the first drug with this mechanism of action that was approved by the FDA for the treatment of the autoimmune disease rheumatoid arthritis.[4] Tofacitinib has been investigated in a wide range of autoimmune diseases including juvenile arthritis, inflammatory bowel disease, and dermatological disorders *e.g.* psoriasis, atopic dermatitis, alopecia and vitiligo.[5-7] Today, five JAK inhibitors (ruxolitinib, tofacitinib, baricitinib, peficitinib, and upadacitinib) are approved as drugs and several more are in late-stage clinical development.[8-11]

Subtype-selective inhibitors are currently in clinical trials with the rationale of maintaining the clinical efficacy of pan-JAK inhibitors while improving safety. In particular, selectivity over JAK2 should have the advantage of avoiding hematological side effect like neutropenia and anemia.[8,9] One of the most advanced selective JAK1 inhibitors, abrocitinib, has been evaluated in Phase III clinical trials in atopic dermatitis with a favorable efficacy and safety profile.[12,13]

We recently reported the discovery of a series of pan-JAK inhibitors that were designed with topical application in mind.[14] The starting point for that work was a fragment screen of a kinase-targeted library of *ca*. 500 fragments screened against JAK2 at a single concentration (100 μM) using SPR. Hits were validated by determination of K_D using SPR and determination of IC_{50} in a biochemical enzyme inhibition assay for JAK1. In general, a good correlation was observed between these two measurements suggesting that most fragments had little or no selectivity for JAK1 over JAK2. However, one compound (**1**) deviated from this trend: its potency towards JAK1 (IC_{50} = 16 μM) was higher than expected from its affinity to JAK2 (K_D = 45 μM). Because of the JAK1 bias and good potency of this unique fragment we selected it as the starting point for the discovery of subtype-selective JAK1 inhibitors reported herein.

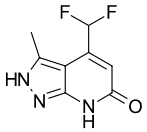
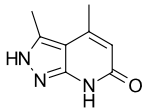
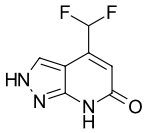
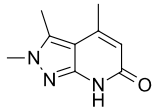
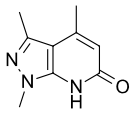
RESULTS AND DISCUSSION

Hit expansion of compound **1** by commercial and synthesized analogs yielded a preliminary map of the SAR of the scaffold (Table 1). Deletion of the fluorine atoms was tolerated with a limited drop in potency (compound **2**), while removal of the pyrazole methyl group was not (compound **3**). Methylation of either of the nitrogen atoms of the pyrazole ring also abolished all activity (compounds **4** and **5**), suggesting that hydrogen bonding interactions with this moiety are important for binding.

In the absence of crystallographic evidence, we assumed that compounds **1** and **2** interact with the kinase hinge

region. In principle, docking should give a good indication of the binding mode of these compounds, but this scaffold can plausibly exist in multiple tautomeric forms, and each of these tautomers would have distinct binding modes. Prediction of the dominant tautomer in solution by quantum mechanical computations was attempted following the method of Ribeiro *et al*,[15] see Table 2. Gas phase (vacuum) molecular geometries were optimized at the M06-2X/6-311G**+ level and were used to calculate solvation energies in water using the SM8 method.[16] Relative energies in water were calculated using the M06-2X functional[17] and two different basis sets, but the results were ambiguous. In vacuum, the 1*H*-pyrazolopyridinol tautomer was strongly favored, but in water, either this tautomer or the 2*H*-pyrazolopyridone tautomer was favored depending on the basis set. Furthermore, the bioactive tautomer may be different from the dominant tautomer in solution, and because of these complications we were unable to rely on docking to determine the binding mode of the scaffold. Instead, a pragmatic approach was taken: given the SAR outlined above, and with the assumption that the scaffold binds to the kinase hinge region, we set out to grow the difluoromethyl substituent of **1** by substituting larger lipophilic moieties that might be able to interact with the ribose pocket of the kinase.

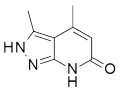
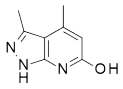
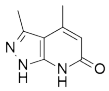
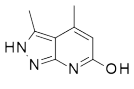
Table 1. Fragment hit and first set of analogs.[a]

Compound	Structure	JAK1 IC_{50} (μM)
1		16
2		30
3		>100
4		>500
5		>900

[a]IC_{50} values were determined in a TR-FRET biochemical assay for kinase inhibition with [ATP] = K_m. All values represent the geometric mean of at least two independent experiments, see Supporting information for details.

Gratifyingly, replacing the difluoromethyl group with a phenyl group (compound **6**) yielded a seven-fold improvement in potency relative to **1**, see Table 3. Because this series originated from our earlier work on non-subtype-selective JAK inhibitors,[14] at the time compound **6** was prepared we had only access to crystallography for JAK2. Because **6** showed a very modest level of selectivity for JAK1 over JAK2, we reasoned that a crystal structure of this compound in complex with JAK2 would be sufficient to establish its binding mode across the JAK kinase family. Thus, an X-ray crystal structure of compound **6** bound to the kinase domain of JAK2 was obtained, see Figure 1. This unequivocally established the bioactive tautomer of the scaffold as the 2*H*-pyrazolopyridone structure (Table 2). Compound **6** engages the kinase hinge region with three hydrogen bonds to the backbone amides of Glu 930 and Leu 932, and a fourth water-mediated hydrogen bond to Pro 933 and the side chain hydroxyl of Tyr 931. Except for the last one, these interactions would be expected to be identical in JAK1 because the only difference in the hinge is Tyr 931 in JAK2 that corresponds to Phe 958 in JAK1. The ligand carbonyl oxygen also nucleates a water network that connects it loosely to the side chain carboxylate of Asp 939 in JAK2. This residue is a glutamic acid (Glu 966) in JAK1 but remains an aspartic acid in JAK3 and TYK2.[18,19] The X-ray structure of compound **6** is consistent with the SAR derived in Table 1. Methylation of either nitrogen of the pyrazole would disrupt the hinge interaction, and deletion of the pyrazole methyl group would remove a productive lipophilic interaction. Methyl group deletion may even create a high energy void that cannot be filled by protein side chain rearrangement or a low energy water molecule. Such effects could explain the dramatic loss of potency of **3** compared to **1**.

Table 2. Tautomers of compound 2 with relative energies in kcal/mol calculated using DFT.[a]

				
Method	2*H*-pyrazolo-pyridone	1*H*-pyrazolo-pyridinol	1*H*-pyrazolo-pyridone	2*H*-pyrazolo-pyridinol
A	2.6	0	4.4	7.4
B	0	0.6	2.8	3.0
C	0.8	0	4.7	3.4

[a]Geometries were optimized in vacuum with the density functional M06-2X[17] and the 6-311G**+ basis set, and these geometries were used for the solvation energy calculations. Methods: A, M06-2X/6-311G**+ in vacuum; B, M06-2X/6-31G* with the SM8 water solvation model; C, M06-2X/6-311G**+ with the SM8 water solvation model.[15,16]

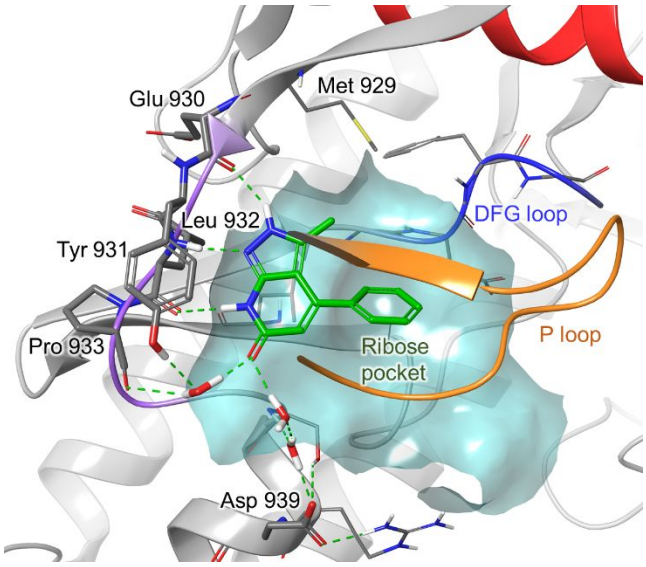


Figure 1. X-ray crystal structure of compound **6** in complex with JAK2 (PDB entry 6TPD, 2.0 Å resolution). The ligand **6** is colored green, the hinge loop is colored purple, the P-loop is colored orange, the DFG loop is colored blue and the αC helix is colored red. Hydrogen bonds are shown as green dotted lines.

With the binding mode and key interactions established, structure-based optimization could be initiated, see Figure 2. The phenyl group of **6** does not appear to make optimal interactions with the lipophilic ribose pocket. Aliphatic moieties with more three-dimensional shape would likely offer a better fit. Thus, the first step should be optimizing this moiety by adding sp^3 carbons and simultaneously keeping lipophilicity at a minimum. The methyl group of **6** seems to fit well, nevertheless, a limited set of analogs with alternative small alkyl groups were targeted to map SAR at this position. The last accessible vector, i.e. the carbon atom next to the carbonyl, points towards the solvent front. It could potentially be exploited to modulate solubility and lipophilicity, and perhaps to further engage Glu 966 (the JAK1 residue corresponding to Asp 939 in JAK2) to improve selectivity for JAK1. Eventually, extending the ligand to contact the P-loop of the kinase was planned to further improve potency.

The biochemical kinase assay used in this work was performed with an ATP concentration close to the K_m of the respective enzyme (JAK1: 7 μM, JAK2: 4 μM, JAK3: 2 μM, TYK2: 13 μM). However, the intracellular concentration of ATP can reach millimolar, and for an ATP-competitive enzyme inhibitor this leads to a dramatic increase in the IC_{50} in a cellular assay compared to the biochemical assay used here. In addition, low permeability across the cell membrane can further reduce or abolish the activity of the tested compound. Thus, cellular STAT reporter gene assays were established for JAK1-STAT6 signaling (to assess JAK1 inhibition) and JAK2-STAT5 signaling (to asses JAK2 inhibition), and compounds were tested in these assays in parallel with the biochemical assays during the optimization campaign reported here.

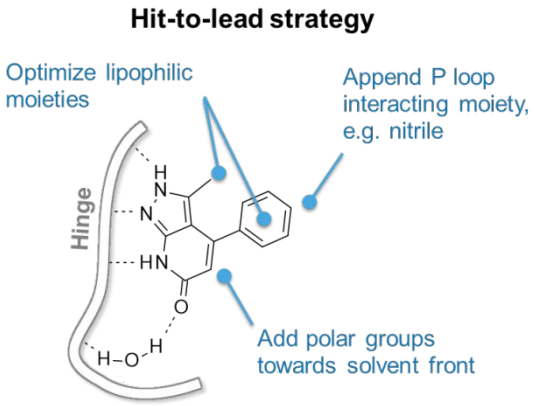


Figure 2. Strategy for lead generation.

To optimize the lipophilic ribose pocket substituent of **6**, the phenyl group was replaced by a selection of mono- and bicyclic aliphatic ring systems, see Table 3. The cyclohexyl analog **7** showed dramatically improved potency and also increased LLE, but very low solubility. This compound was the first with activity in the cellular STAT6 assay. Importantly, compound **7** exhibited a 10-fold selectivity for JAK1 over JAK2 in the biochemical assays. Although the reason for this jump in selectivity relative to compound **6** is unclear, this result prompted a thorough investigation into this chemical series. Smaller, less lipophilic substituents e.g. cyclopentyl (**8**) and cyclobutyl methyl (**9**) afforded similar levels of potency with improved LLE and solubility. The racemic *cis*-methyl cyclohexyl analog **10** possessed improved potency in both enzymatic and cellular assays, albeit with a significant log D penalty. The design of this compound was inspired by the similar positioning of the methyl group of tofacitinib (**20**),[4,20] see Figure 3 and the discussion below. Racemic norbornane analogs **11** and **12** showed similar or even higher potency with slightly reduced log D and thus improved LLE with the same number of sp^3 carbons as **10**. Importantly, compared with **7**, compounds **11** and **12** achieved a four-fold improvement in cellular potency with no increase in lipophilicity and much improved solubility. The racemic bicyclo[2.2.2]octane analog **13** displayed no further improvement in potency, and its increased lipophilicity caused a drop in LLE and solubility. Thus, the norbornanes **11** and **12** were the best performing analogs as they combined excellent potency with LLE on par with the smaller, less potent analogs **8** and **9**. The log D advantage of bicyclic over monocyclic scaffolds has been noted for bicyclic morpholine and piperazine analogs, although the effects for those compounds are partly due to increased basicity.[21] Synthetic access to norbornane analogs of specific stereochemistry is restricted, so for synthetic expedience, we initially chose to optimize the cyclohexane derivative **7**. This would allow us to explore substitution towards the solvent front and the P-loop in detail, and if necessary, we envisaged that optimized substituents could be transferred back to the norbornane analogs.

As noted above, a methyl substituent on the pyrazole appeared optimal based on the X-ray structure of **6**, but a limited exploration seemed justified to confirm this hypothesis. Deletion of the methyl group in **7** yielded a

compound (**14**) with measurable, but ~110-fold reduced JAK1 potency, thus confirming the importance of this substituent (compare compounds **1** and **3** discussed above). Opposing trends in the enzymatic and cellular assays were observed for the series methyl (**7**), ethyl (**15**) and cyclopropyl (**16**), perhaps reflecting improved permeability of the more lipophilic analogs. The increased cellular potency of **15** and **16** was negated by increased lipophilicity as the LLE was unchanged compared with **7**. Thus, the methyl group was retained at this position throughout the rest of the optimization.

The only unsubstituted carbon atom of the core scaffold offered a vector towards the solvent front and the selectivity-modulating residue Glu 966, and this position was targeted next. A hydroxymethyl substituent (**17**) was tolerated: no major difference in cellular and enzymatic potency compared to **7** was observed, and LLE and solubility were improved. Interestingly, **17** exhibited a two-fold improvement in selectivity over JAK2 compared to **7**, suggesting a productive interaction with Glu 966 of JAK1 or a disruption of the water network connecting the ligand to Asp 939 in JAK2. Thus, we noted that a hydroxymethyl substituent might be useful later in the optimization campaign, should we need to solve issues resulting from high log D, e.g. low solubility or poor metabolic stability. Hydroxyethyl (**18**) and cyano (**19**) analogs were less potent and were not considered further.

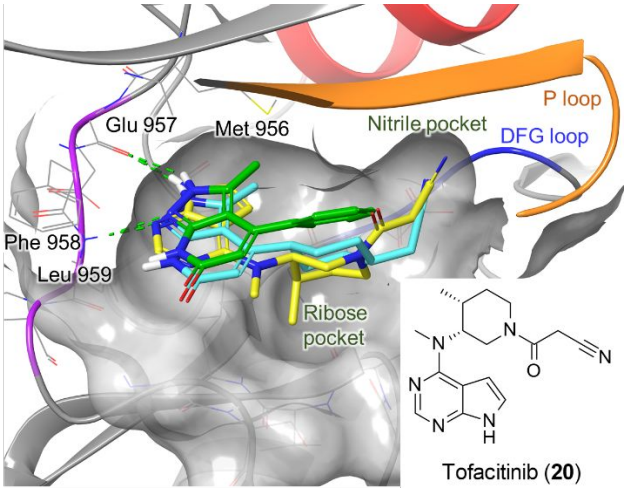


Figure 3. Docking pose of **21** (cyan) overlaid with the X-ray structures of **6** (green) bound to JAK2 (PDB entry 6TPD) and tofacitinib **20** (yellow) bound to JAK1 (PDB entry 3EYG). The protein from PDB entry 3EYG is shown, and this was used to dock **21**. The hinge loop is colored purple, the P-loop is colored orange, the DFG loop is colored blue and the αC helix is colored red. Hydrogen bonds are shown as green dotted lines.

Having optimized the lipophilic substituents of the core, we proceeded to grow the prototypical analog **7** towards the P-loop. In the spirit of fragment-based drug design – start small and make every added atom count – small substituents with balanced polarity were added to the cyclohexyl analog **7**, see Table 4. To direct the substituent towards the P-loop while avoiding the complication of fluctuation between two axial/equatorial chair conformers, only *trans*-configured substituents at the 4-position of the cyclohexyl ring were considered. With the bioactive tautomer and binding mode known, docking could now be leveraged to inform the design of analogs. One example is shown in Figure 3. Here, the published X-ray crystal structure of the JAK inhibitor tofacitinib (**20**)[20] overlaid on the X-ray structure of **6** (Figure 1) suggested that an acetonitrile appended to compound **7** might overlay with the acetonitrile moiety of tofacitinib. Indeed, docking this hypothetical compound **21** into the tofacitinib X-ray structure indicated a fairly close overlay of the nitriles in the two compounds, see Figure 3. The reported SAR of tofacitinib (**20**) indicates that its nitrile moiety contributes significantly to the binding affinity.[4] Thus, we synthesized and tested compound **21**, and it afforded a three-fold improvement in cellular potency relative to **7** with a concomitant increase in LLE from 2.6 to 4.4, see Table 4. Because the nitrile of **21** appears to be slightly further from the P-loop than the corresponding nitrile in **20** (see Figure 3), a one-carbon homolog, **22**, was prepared to try to reach closer towards the P-loop, but potency was essentially unchanged. On the other hand, contracting the nitrile back to the cyclohexane caused a large drop in activity because this compound (**23**) cannot reach the P-loop and the nitrile does not add other interactions with the kinase. The desolvation penalty of the nitrile may explain the six-fold drop in potency relative to the unsubstituted cyclohexane **7**.

To better understand the postulated interaction between the nitrile of **21** and the P-loop of the kinase, an X-ray crystal structure of **21** in complex with the JAK1 kinase domain was solved, see Figure 4. The core scaffold and cyclohexane moieties in the crystal structure overlaid perfectly with the predicted binding mode from docking (Figure 3). However, the nitrile functional group was not defined by the electron density suggesting that it adopts several conformations. Thus, it is unlikely that it engages the P-loop like the nitrile in tofacitinib (**20**), which has a clearly defined electron density (PDB entry 3EYG). With this result in hand we set out to investigate other options for reaching the P-loop and forming productive interactions with it. To this end, compounds with amide, ester and sulfonamide linkers connecting the cyclohexane moiety with small cycloalkyl and fluorocycloalkyl moieties were synthesized (Table 4). The cyclohexyl carboxamide **24** possessed modest JAK1 potency, slightly lower than for **7**, while the potency of reversed amide **25** was much lower. Increasing the steric bulk of **24** to cyclopentane **26** only afforded a two-fold improvement. Thus, **26** was no better than **7** with respect to JAK1 potency. Gratifyingly, introduction of a *gem*-difluoro motif to **24** greatly improved JAK1 potency, particularly in the *S* enantiomer **27**, but the less active *R* enantiomer **28** also showed a slight improvement relative to **24**.

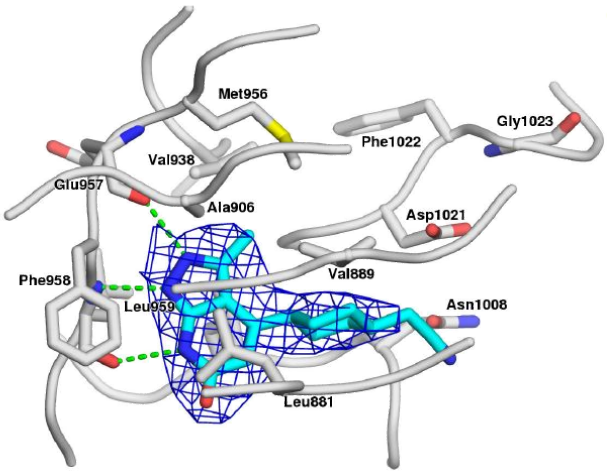
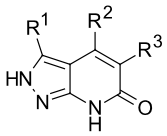
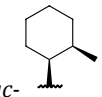


Figure 4. X-ray crystal structure of compound **21** (cyan) in complex with JAK1 (PDB entry 6TPE, 2.9 Å resolution). The ligand electron density is shown in blue mesh. Hydrogen bonds are shown as green dotted lines. The nitrile functional group is modelled in an arbitrary rotameric state, because no electron density was observed for it.

Unfortunately, the high potency of **27** in the enzymatic assay did not translate to the cellular STAT6 assay. For the compounds discussed up to this point, on average a *ca*. 30-fold drop in potency was observed going from enzymatic to cellular assays, largely consistent with the different ATP concentrations in these assays as discussed above. However, for compound **27**, the drop was close to 3,000-fold, suggesting a permeability limitation for this analog. We suspected that the polar nature of the scaffold core together with the additional hydrogen bond donor of **27** might be responsible for the putative permeability limitation. To remove one hydrogen bond donor, the ester analog **29** was prepared and tested. This compound retained most of the potency in the JAK1 assay and showed a potency in the STAT6 assay consistent with the expected 30-fold drop discussed above. However, this compound had poor solubility and was extremely rapidly degraded in human liver microsomes, presumably due to ester hydrolysis. Replacing the amide linker with a sulfonamide was briefly investigated (compounds **30** and **31**) but did not result in an improvement in potency or lower drop in activity from biochemical to cellular assays. For completeness, we tested the building blocks **32** and **33** used to prepare compounds **24** and **26**-**31**, but they were much less potent than **7**. Very large desolvation penalties of the hydroxy and amino groups of these compounds in the absence of productive interactions with the protein may explain this result.

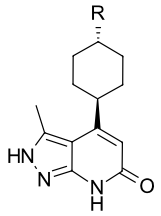
Table 3. Optimization of the lipophilic substituents.[a]

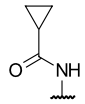
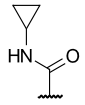
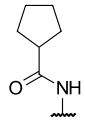
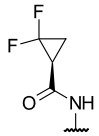
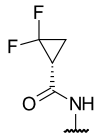
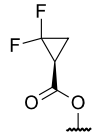
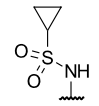
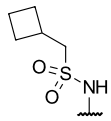


Cpd	R^1	R^2	R^3	STAT6 IC_{50} (μM)	STAT5 IC_{50} (μM)	JAK1 IC_{50} (μM)	JAK2 IC_{50} (μM)	JAK3 IC_{50} (μM)	TYK2 IC_{50} (μM)	Log $D_{7.4}$[b]	Aq. sol. (μM)[b]	STAT6 LLE
6	Me	Ph	H	>50	n.d.	2.2	4.2[b]	2.6[b]	3.9[b]	2.2	>90	<2.1 3.5[c]
7	Me	cyclohexyl	H	3.7	>40	0.13	1.3	0.55	0.67	2.8	4	2.6 4.1[c]
8	Me	cyclopentyl	H	4.3	>40	0.072	1.3	0.67	0.83	2.2	39	3.2
9	Me		H	3.4	>40	0.12	0.57	0.48	1.1	2.2	16	3.3
10	Me	*rac-* 	H	1.3	8.9	0.040	0.34	0.11	0.70	3.0	8	2.9
11	Me	*rac-* 	H	1.0	>40	0.044	0.38	0.18	0.15	2.7	33	3.3
12	Me	*rac-* 	H	0.85	22	0.046	0.43	0.24	0.43	2.8	64	3.3
13	Me	*rac-* 	H	0.89	9.1	0.054	0.33	0.065	0.22	3.3	8	2.7
14	H	cyclohexyl	H	>50	n.d.	15	35[b]	12[b]	24[b]	n.d.	n.d.	n.d.
15	Et	cyclohexyl	H	2.5	>40	0.46	2.7	1.6	n.d.	3.0	n.d.	2.6
16	*c*-Pr	cyclohexyl	H	1.8	>40	0.50	1.3	0.98	1.6[b]	3.1	n.d.	2.6
17	Me	cyclohexyl	CH_2OH	4.8	>40	0.14	2.8	1.4	0.65	2.1	100	3.2
18	Me	cyclohexyl	CH_2CH_2OH	8.5	>40	0.49	3.9	0.99	3.4	2.6	11	2.5
19	Me	cyclohexyl	CN	20[b]	>40	2.1	17	5.4	11	n.d.	n.d.	n.d.

[a]STAT IC_{50} values were determined in a cellular reporter gene assay and JAK IC_{50} values were determined in a TR-FRET biochemical assay for kinase inhibition with [ATP] = K_m. All values represent the geometric mean of at least two independent experiments unless otherwise noted, see Supporting information for details. Log $D_{7.4}$ was determined using a shake flask assay and aqueous solubility was determined using an HPLC assay from DMSO stock solutions of test compounds diluted in phosphate buffer at pH 7.4. [b]Only one determination was made. [c]LLE based on JAK1 IC_{50}.

Table 4. Addition of polar substituents projecting towards the P-loop.[a]



Cpd	R	STAT6 IC_{50} (μM)	STAT5 IC_{50} (μM)	JAK1 IC_{50} (μM)	JAK2 IC_{50} (μM)	JAK3 IC_{50} (μM)	TYK2 IC_{50} (μM)	Log $D_{7.4}$[b]	Aq. sol. (μM)[b]	STAT6 LLE
21	CH_2CN	1.2	>40	0.040	0.36	0.27	0.13	1.5	59	4.4
22	CH_2CH_2CN	1.2	13	0.032	0.16	0.28	n.d.	1.8	18	4.1
23	CN	n.d.	n.d.	0.73	5.1	11	n.d.	n.d.	n.d.	n.d.
24		>14	>14	0.22	2.1	7.0	2.8[b]	1.2	22	<3.7
25		>40	>40	2.0	9.4	1.1	14[b]	n.d.	n.d.	n.d.
26		9.1	>30	0.11	1.6	4.8	6.8[b]	n.d.	n.d.	n.d.
27		1.7	>7	0.0064	0.12	0.48	0.22[b]	1.3	43	4.5
28		>40	>40	0.078	1.7	2.7	2.7[b]	1.3	29	<3.1
29		0.42	>25	0.014	0.12	0.091	n.d.	n.d.	3	n.d.
30		n.d.	n.d.	0.40	2.5	6.6	n.d.	n.d.	n.d.	n.d.
31		3.2	>10	0.040	0.57	1.7	n.d.	n.d.	n.d.	n.d.
32	NH_2	>40	>40	0.82	18	11	16[b]	n.d.	n.d.	n.d.
33	OH	20	>40	0.49	4.6	1.7	4.2	0.8	>85	3.8

[a]STAT IC_{50} values were determined in a cellular reporter gene assay and JAK IC_{50} values were determined in a TR-FRET biochemical assay for kinase inhibition with [ATP] = K_m. All values represent the geometric mean of at least two independent experiments unless otherwise noted, see Supporting information for details. Log $D_{7.4}$ was determined using a shake flask assay and aqueous solubility was determined using an HPLC assay from DMSO stock solutions of test compounds diluted in phosphate buffer at pH 7.4. [b]Only one determination was made.

To confirm the binding mode of **27** and understand the details of its interaction with the JAK1 kinase domain, an X-ray crystal structure of this compound in complex with JAK1 was solved, see Figure 5. The pyrazolopyridone core makes the same interactions with the hinge loop residues as do compounds **6** and **21**. The higher resolution of this X-ray structure allows the water network to be observed. A water-mediated contact with Arg 879 and Pro 960 is observed, but no interaction with Glu 966 is seen. Moreover, the two protein molecules in the crystallographic unit cell show different side chain conformations for this residue. In contrast, the Arg 879 side chain has a very similar conformation in both protein molecules. This suggests that the JAK1 selectivity of the scaffold derives from the interaction with Arg 879. This residue is Gln in JAK2, Ser in JAK3, and Arg in TYK2. However, the combination of Phe 958 (Tyr in JAK2, JAK3 and TYK2) and Arg 879 is unique to JAK1. Comparing the binding modes of **6** in JAK2 and **27** in JAK1 may give a clue to the reason for the JAK1 selectivity of the scaffold: The carbonyl oxygen of **6** (Figure 1) makes a water-mediated contact with the JAK2 Tyr 931 hydroxyl while **27** makes a very similar contact with the JAK1 Arg 879 side chain. If the latter interaction is more favorable, that would explain the preference of the scaffold for JAK1 binding.

Other strategies for achieving high selectivity for JAK1 have been pursued in the literature, and a large body of work by many groups has been reviewed.[18,19] In particular, targeting Glu 966 in JAK1 has been shown to be a viable approach, although other amino acid differences between the JAK family subtype enzymes have also been exploited. For a comprehensive discussion of these approaches, see Reference 19. Alternatively, it has been suggested that the P-loop is more flexible in JAK1 than in JAK2. Extending the ligand towards the P-loop thus causes clash with this moiety in JAK2, thus rendering compounds JAK1-selective.[12] Although, as noted above, interaction with Glu 966 seems unlikely to contribute to the selectivity of the series reported herein, a contribution from the differential P-loop flexibility may well augment the inherent JAK1 bias of compound **27**.

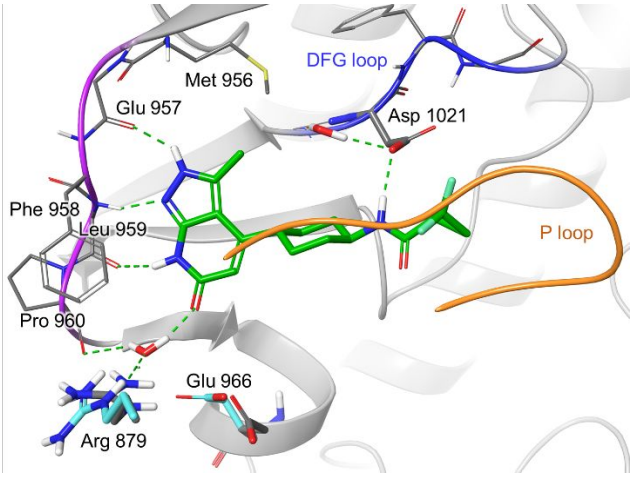
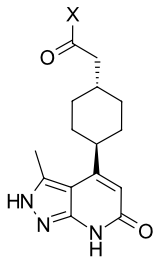


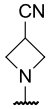
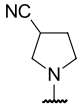
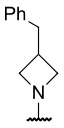
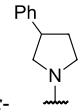
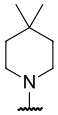
Figure 5. X-ray crystal structure of compound **27** in complex with JAK1. (PDB entry 6TPF, 2.3 Å resolution). The ligand **27** is colored green, the hinge loop is colored purple, the P-loop is colored orange, and the DFG loop is colored blue. Hydrogen bonds are shown as green dotted lines. Side chain conformations from the second protein molecule in the crystallographic unit cell are shown in cyan for Arg 879 and Glu 966.

The *gem*-difluoro moiety of **27** does contact the P-loop, however the reason for the 34-fold increase in potency observed when adding these two fluorine atoms to **24** is not obvious from visual inspection of the X-ray structure. A hydrogen bonding interaction between the amide NH and the backbone carbonyl of the Asp 1021 residue of the DFG loop is present, but it probably contributes little to the binding affinity, considering that the ester analog **29** is almost as potent. Thus, to improve permeability by removing one hydrogen bond donor, the amide NH of **27** was replaced by CH_2 and a tertiary amide nitrogen was introduced on the other side of the carbonyl, see Table 5. With this change, the direct analog of **27** would be an aziridine amide, which would be expected to have poor chemical stability. Thus, we targeted azetidine, pyrrolidine and piperidine amides instead.

To maintain lipophilicity under control, the nitrile functional group was re-introduced as a P-loop interaction moiety. Both azetidine **34** and pyrrolidine **35** showed respectable levels of JAK1 potency, but unfortunately cellular potency was not achieved. It is worth noting that **35** is racemic, and the pure enantiomers were not prepared because of the lack of sufficient cellular activity. Both compounds possessed good aqueous solubility and were stable towards human liver microsomes. To improve cellular potency at the expense of increasing log D, the nitrile was replaced by more lipophilic substituents, e.g. benzyl (**36**), phenyl (**37**) and *gem*-dimethyl (**38**). Although these compounds showed excellent JAK1 potency and cellular potency in the STAT6 assay, consistent with the expected 30-fold drop, solubility and in particular metabolic stability were very poor. Clearly, the polar nature of the core scaffold limits the amount of additional polarity that can be tolerated in the compounds without losing cellular potency. On the other hand, compounds with sufficient lipophilicity to achieve cellular activity showed poor solubility and metabolic stability.

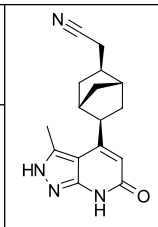
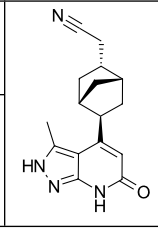
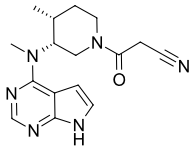
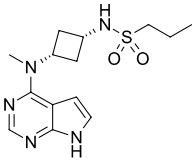
Table 5. Tertiary amides projecting towards the P-loop.[a]



Cpd	X	STAT6 IC_{50} (μM)	STAT5 IC_{50} (μM)	JAK1 IC_{50} (μM)	JAK2 IC_{50} (μM)	JAK3 IC_{50} (μM)	Log $D_{7.4}$[b]	Aq. sol. (μM)[b]	HLM Cl_{app} (ml/min/kg)[b]	STAT6 LLE
34		>30	>30	0.013	0.064	0.097	0.5	540	<8	<4.0
35	 *rac-*	14	>45	0.011	0.089	0.15	0.7	85	<8	4.2
36		0.25	5.1	0.0030	0.015	0.088	3.0	2.4	87	3.6
37	 *rac-*	0.11	2.4	0.0021	0.019	0.063	3.0	n.d.	75	4.0
38		0.11	3.4	0.0034	0.032	0.057	2.9	n.d.	70	4.0

[a]STAT IC_{50} values were determined in a cellular reporter gene assay and JAK IC_{50} values were determined in a TR-FRET biochemical assay for kinase inhibition with [ATP] = K_m. All values represent the geometric mean of at least two independent experiments, see Supporting information for details. Log $D_{7.4}$ was determined using a shake flask assay and aqueous solubility was determined using an HPLC assay from DMSO stock solutions of test compounds diluted in phosphate buffer at pH 7.4. HLM Cl_{app} is the intrinsic clearance in human liver microsomes. [b]Only one determination was made.

Table 6. Norbornane nitriles compared to benchmark pan-JAK and selective JAK1 inhibitors.[a]

Compound		STAT6 IC_{50} (μM)	STAT5 IC_{50} (μM)	JAK1 IC_{50} (μM)	JAK2 IC_{50} (μM)	JAK3 IC_{50} (μM)	TYK2 IC_{50} (μM)	Log $D_{7.4}$[b]	Aq. sol. (μM)[b]	HLM Cl_{app} (ml/min/kg)[b]	STAT6 LLE
39		0.77	15	0.011	0.093	0.42	0.18	n.d.	n.d.	n.d.	n.d.
40		0.077	2.8	0.0010	0.012	0.088	0.020	1.5	89	<8	5.6
41		1.2	24	0.030	0.15	0.22	0.22	1.5	>92	<8	4.4
42		2.7	>43	0.047	0.36	0.97	0.33	1.6	290	<8	4.0
Tofacitinib (**20**)		0.087	0.12	0.0031	0.0027	0.0015	0.072	1.9	n.d.	<8	5.2
Abrocitinib		0.18	1.4	0.0051	0.036	0.48	0.16	2.0	n.d.	<8	4.7

[a]Compounds **39** and **40** are enantiomers of unknown absolute configurations, as are compounds **41** and **42**. STAT IC_{50} values were determined in a cellular reporter gene assay and JAK IC_{50} values were determined in a TR-FRET biochemical assay for kinase inhibition with [ATP] = K_m. All values represent the geometric mean of at least two independent experiments, see Supporting information for details. Log $D_{7.4}$ was determined using a shake flask assay and aqueous solubility was determined using an HPLC assay from DMSO stock solutions of test compounds diluted in phosphate buffer at pH 7.4. HLM Cl_{app} is the intrinsic clearance in human liver microsomes. [b]Only one determination was made.

To overcome these issues, we set out to transfer the best P-loop-targeting substituents discovered above back to the norbornane scaffold (compounds **11** and **12**). Thus, we hoped to significantly improve the potency of compound **21** without compromising solubility and metabolic stability. Although no difference in potency between the unsubstituted *exo* (**11**) and *endo* (**12**) norbornanes was observed, once a second substituent is appended to the norbornane this must be directed towards the P-loop to achieve the desired potency boost. Although docking could not conclusively direct our efforts towards a single regio- and stereoisomer, it appeared unlikely that the core hinge binder could occupy an endo vector. To closely mimic the arrangement of the core and nitrile of **21**, the four stereoisomers **39**-**42** were targeted for synthesis, see Table 6. The compounds were prepared as racemates and the enantiomers were separated by chiral chromatography. The absolute configurations of these compounds were not determined. Gratifyingly, one enantiomer of the *exo*,*exo* diastereomer, compound **40**, afforded high enzymatic and cellular potency combined with high metabolic stability in human liver microsomes and good solubility. Importantly, **40** showed excellent selectivity for JAK1 over JAK2 in both assay formats: In the enzymatic assays with ATP at K_m, a 12-fold selectivity was found, and in the STAT cellular assays, a 36-fold selectivity was observed. The enantiomer of **40**, i.e. compound **39**, and the enantiomeric pair of *endo*,*exo* diastereomers **41** and **42** all showed much lower potency.

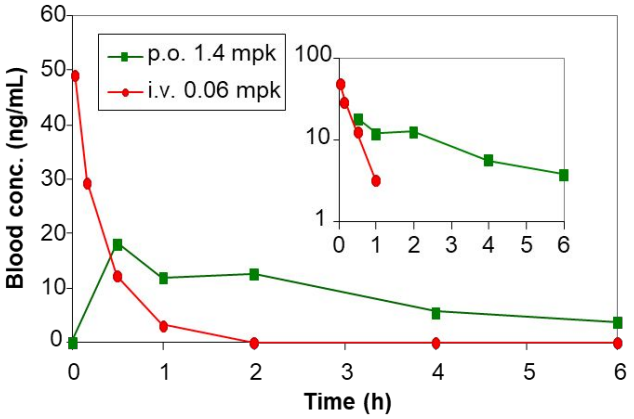


Figure 6. Rat PK study with compound **40**. $T_{½}$ = 0.3 h, Cl = 53 mL/min/kg, V_{β} = 1.2 L/kg, F = 14%. Inset: Blood concentration on a logarithmic scale vs. time.

To benchmark the selectivity profile of compound **40**, we assayed the pan-JAK inhibitor tofacitinib (**20**)[4] and the selective JAK1 inhibitor abrocitinib[12] that recently completed Phase III clinical trials in atopic dermatitis.[13] As expected, tofacitinib (**20**) showed no selectivity for JAK1 over JAK2, nor did it show any selectivity in the cellular STAT reporter gene assays. Abrocitinib on the other hand showed a 7-fold selectivity for JAK1 over JAK2, and 8-fold selectivity in the cellular STAT reporter gene assays.

Compound **40** was selected for further profiling to assess its potential as a lead compound. In a panel of 50 kinases broadly covering the kinome it only inhibited one kinase more than 30% (FGFR1: 32%) when tested at 1 μM. All assays in the panel were conducted with 1 mM ATP, see Supporting Information for details. Compound **40** did not inhibit the cytochrome P450 enzymes Cyp1A2, 2C9, 2C19, 2D6, or 3A4 up to 25 μM and showed no effect in a hERG binding assay at 30 μM. Good *in vitro* metabolic stability was found in rat liver microsomes (Cl_{app} < 15 mL/min/kg) and rat hepatocytes (Cl_{app} = 25 mL/min/kg), and rat plasma protein binding was moderate (f_u = 22%). A low permeability (P_{app} = 0.7 × 10^{-6} cm/s) and a high efflux ratio = 40 was observed in the Caco-2 assay suggesting a potential permeability limitation for oral absorption from the gut. Thus, compound **40** was tested in an early discovery rat PK study (only one animal for each route of administration), see Figure 6, and was found to exhibit high clearance, short half-life and fairly low oral bioavailability (F = 14%). Assuming no elimination in the gut, the fraction of dose absorbed (F_{abs}) was calculated to approx. 41%. This confirms that **40** has a permeability-limited absorption as suggested by the Caco-2 data. The *in vitro* to *in vivo* extrapolation using the well stirred model adjusted for protein binding was poor: The predicted *in vivo* unbound clearance based on rat microsomal or hepatocyte intrinsic clearance was <2.0 and 10 mL/min/kg, respectively, indicating extrahepatic clearance mechanisms.

CONCLUSIONS

A fragment screening hit **1** was developed into lead compound **40** using a structure-guided fragment growing approach. Throughout the lead generation campaign, a strict focus was kept on adding the minimum amount of molecular weight and lipophilicity at every step. During the progression from **1** to **40**, the molecular weight increased from 199 to 282, the number of non-hydrogen atoms increased from 14 to 21, and JAK1 potency increased 16,000-fold (4.2 log units). The resulting lead compound **40** displayed potency in the cellular STAT6 assay below 100 nM, 36-fold selectivity over JAK2 signalling as measured in the cellular STAT assays, and physicochemical properties that satisfied our lead compound criteria.

CHEMISTRY

To support the fragment-based lead generation campaign reported above, a flexible and convenient synthetic route to the 2,7-dihydropyrazolo[3,4-*b*]pyridin-6-one scaffold was needed. Synthetic routes to this scaffold have been reviewed[22] and our initial attempt at re-synthesis of compound **1** was based on the condensation between 3-amino-5-methylpyrazole (**43c**) and ethyl difluoroacetoacetate (**49**) in acetic acid, see Scheme 1. Based on the reported regiochemical outcome of reactions of 3-aminopyrazoles (**43a** and **43b**) and ethyl trifluoroacetoacetate (**44**) that yielded **45** and **46** respectively,[23-25] we expected to obtain **1**. However, the product of this reaction was not identical to the commercial sample of **1** and was inactive in the JAK1 assay. Based on ^{1}H-^{13}C HMBC NMR and predicted ^{13}C chemical shifts we assigned structure **50** to the product, see supporting information for details. This result is contrary to the reported result that only when the 4-position was blocked was this regiochemistry observed, e.g. pyrazole **47** was reported to give **48** when reacted with **44**.[23] To avoid the formation of **50**, the pyrazole NH position was protected with a *t*-Bu group.[26] Reaction of the protected pyrazole **51** with **49** afforded **55**, which upon deprotection with TFA yielded **1** identical to the commercial sample and with the same JAK1 potency. Compounds **3**, **6** and **14** were similarly prepared starting from the appropriate pyrazoles (**51** and **52**) and β-ketoesters (**49**, **53** and **54**).

Although a regiochemically unambiguous route was now available, the harsh conditions needed for both the cyclo-condensation and the deprotection steps, and the necessity of constructing β-keto ester starting materials lead us to explore alternatives. A three-component reaction between an aminopyrazole (**43**), an aldehyde (**59**) and Meldrum's acid (**60**) to afford intermediate **61**[27,28] appeared attractive, see Scheme 2. When aromatic α-keto aldehydes were employed, air oxidation of **61** in either acidic or basic media furnished the desired 2,7-dihydropyrazolo[3,4-*b*]pyridin-6-ones.[28] In our hands, air oxidation of **61** was inefficient with aliphatic R^2 groups, but oxidation with DDQ readily afforded the desired products under mild conditions. This method allowed access to the target compounds in two steps from readily available starting materials and was used to prepare all final compounds reported herein except **1**-**6**, **14** and **19**. In many cases we observed the formation of a by-product (**61p**) in the three-component reaction. Compound **61p** is presumably

formed from in situ generated acetone deriving from Meldrum's acid (**60**). The formation of **61p** from **43a**, **60** and acetone in refluxing methanol has been reported in the literature.[29] In some cases, **61p** was the only product formed when the reaction was carried out in ethanol. Switching solvent to pyridine with 10 mol% piperidine for the condensation of aldehyde **59** with Meldrum's acid **60** followed by addition of pyrazole **43c** and ethanol allowed formation of the desired product in those problematic cases (**61c** and **61n**).

To access compounds **17**-**19** with the carbonyl α-carbon functionalized, two methods were used. Compounds **17** and **18** were prepared from intermediate **61a** by simultaneous chlorination of the amide functionality and Vilsmeier-Haack formylation of the α-carbon as shown in Scheme 3. The resulting chloride **62** was converted to the corresponding methoxide **63**, which was reduced to alcohol **64** without isolation and demethylated to yield **17**. Alternatively, methoxide **63** was subjected to a Wittig reaction yielding alkene **65**. Hydroboration-oxidation then afforded alcohol **66** which was demethylated to give **18**. To prepare compound **19**, a cyclocondensation between aminopyrazole **43c** and malononitrile **67** was employed to yield intermediate **68**, see Scheme 4. Oxidation by bromination followed by elimination of HBr afforded compound **19**.

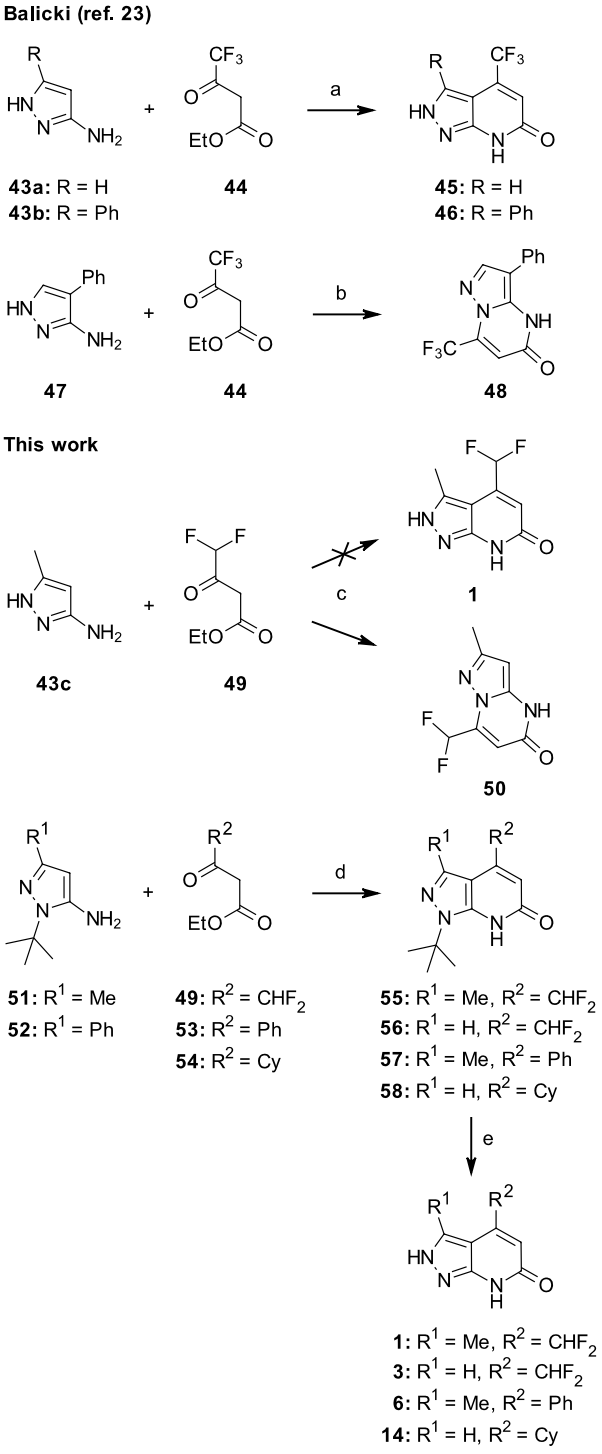
Nitrile **22** was prepared by homologation of the nitrile intermediate **61j**, see Scheme 5. Protection of the NH protons with 4-methoxybenzyl chloride gave **69**, which appeared to be a single regioisomer according to the ^{1}H NMR spectrum. The regiochemistry of the PMB groups was not investigated. Structure **69** and the following structures show one plausible regioisomer, but no attempt to determine if this is the correct one was made. Oxidation of **69** with DDQ gave **70**, which upon reduction with DIBAL-H yielded aldehyde **71**. This was further reduced to the alcohol **72**, mesylated to yield **73**, and treated with NaCN to afford the homologated nitrile **74**. Deprotection in refluxing TFA finally yielded compound **22**.

The syntheses of the amides **24**, **26**-**28**, sulfonamides **30** and **31**, and ester **29** are outlined in Scheme 6. The amides were prepared by HATU coupling of amine **32** with the respective acids, and the sulfonamides were prepared by reacting **32** with the respective sulfonyl chlorides. The ester **29** was prepared by HATU/DMAP coupling of alcohol **33** with (1*S*)-2,2-difluorocyclopropanecarboxylic acid. To prepare the inverted amide **25**, the route in Scheme 7 was used. Protection of the ester intermediate **61n** afforded **75**, which was oxidized with DDQ to afford **76**, which appeared to be a single regioisomer according to the ^{1}H NMR spectrum (see the discussion of **69** above). Hydrolysis of the methyl ester yielded acid **77** and amide coupling with HATU followed by deprotection gave compound **25**. Amides **34**-**38** were prepared in a library format from acid intermediate **78** and the respective amines, see Scheme 8.

The acetonitrile norbornanes **39**-**42** were prepared from the common intermediate **39a**, see Scheme 9. Compound **39a** is a mixture of four stereoisomers: it consists of the enantiomeric pairs of the (*Z*)-olefin/*exo*-norbornane and (*E*)-olefin/*exo*-norbornane diastereomers in a ca. 1:1 diastereomeric ratio. Hydrogenation of the olefin thus afforded a ca. 1:1 mixture of racemic *exo*,*exo*-norbornane and *endo*,*exo*-norbornane diastereomers. This mixture was subjected to chiral SFC separation to afford the four stereoisomers **39**-**42** individually. Relative stereochemistry was assigned from ^{1}H NMR NOE (see Supporting information for details) but no attempt to assign absolute configuration was made. Thus, although **39** and **40** are enantiomers, the assignment of absolute configuration shown in Scheme 9 is arbitrary. This also holds for **41** and **42**.

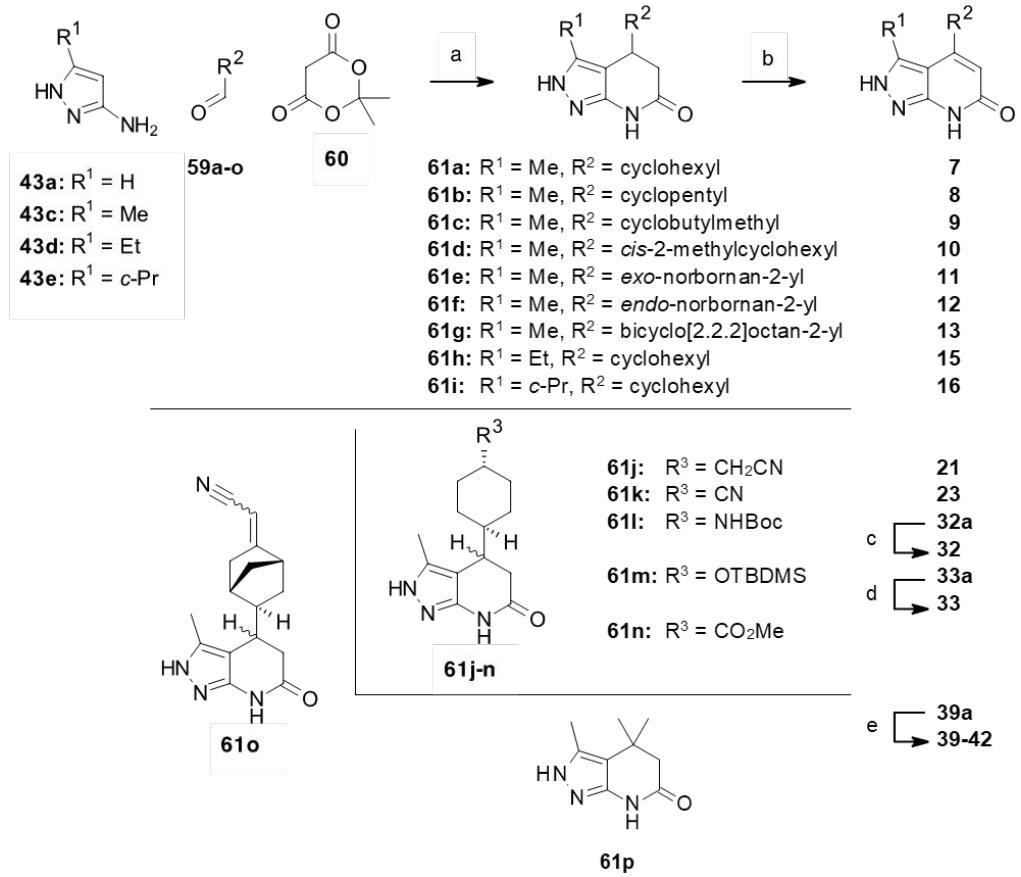
Although most of the aldehydes **59** (see Scheme 2) required to prepare the final compounds were commercially available, **59d**-**f**, **59j** and **59o** needed to be synthesized. The routes used to access these intermediates are shown in schemes 10-13. Starting from the known ester **79**,[30] the racemic aldehyde **59d** was prepared by $LiAlH_4$ reduction to furnish alcohol **80**, which was oxidized with Dess-Martin periodinane (Scheme 10). The norbornanecarbaldehydes **59e** and **59f** were prepared from commercially available norbornene carboxylic acid **81** (*exo*/*endo* mixture, predominantly *endo*) as shown in Scheme 11.[31] After esterification with isopropanol, the *exo* and *endo* esters **82** and **83** could be separated by silica gel column chromatography. The two isomers were separately treated with $LiAlH_4$ to afford alcohols **84** and **86**, which were hydrogenated to afford norbornane methanols **85** and **87**. Swern oxidation furnished the required aldehyde building blocks **59e** and **59f**. To synthesize building block **59j** the route in Scheme 12 was employed. Mesylation of alcohol **88** afforded intermediate **89**, which was treated with KCN to yield nitrile **90**. Chemoselective reduction of the ester moiety with $LiBH_4$ gave alcohol **91**, which upon Swern oxidation furnished aldehyde **59j**. The route to aldehyde **59o** is outlined in Scheme 13. Analogous to the preparation of esters **82** and **83** (Scheme 11), the corresponding benzyl esters **92** and **93** were prepared from norbornene carboxylic acid **81** and separated using silica gel column chromatography. The *exo* isomer **93** was subjected to hydroboration/oxidation to afford a separable mixture of regioisomers **94** and **95**.[32] Dess-Martin periodinane oxidation of the desired regioisomer **94** afforded ketone **96**, which was converted to alkene **97** by means of a HWE olefination. Chemoselective reduction of the ester moiety with $LiBH_4$ gave alcohol **98**, which upon Swern oxidation furnished aldehyde **59o**.

Scheme 1. First route to the pyrazolopyridone scaffold[a]



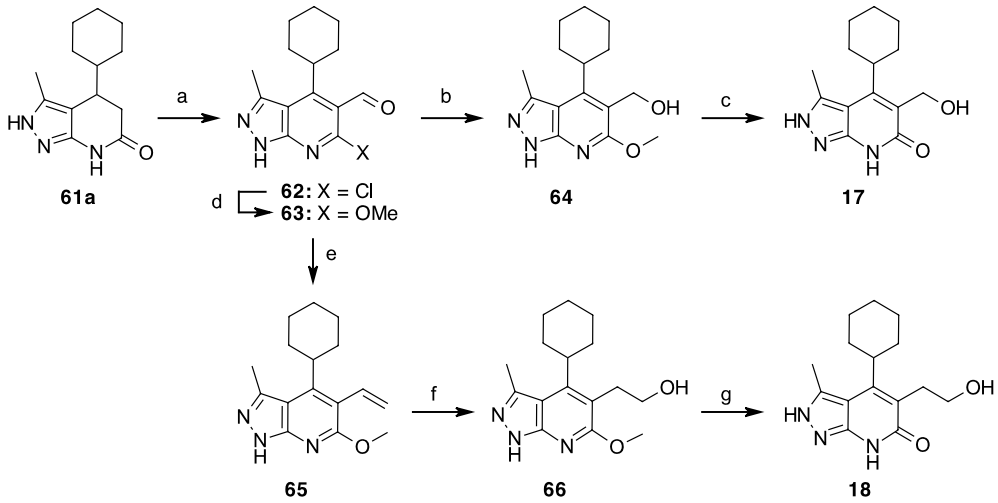
[a]Reagents and conditions: (a) AcOH, reflux, 76–82%; (b) AcOH, reflux, 88%; (c) AcOH, 135 °C (microwave heating), 23%; (d) AcOH, 110 °C, 12–98%; (e) TFA, 70 °C, 16–68%.

Scheme 2. Three-component reaction route to the pyrazolopyridone scaffold[a]



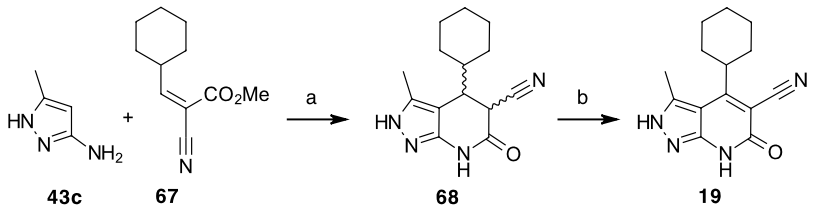
[a]Reagents and conditions: (a) EtOH, 60–70 °C or reflux, 23–86%; (b) DDQ, 1,4-dioxane, 100 °C, 12–87%; (c) 4 M HCl in 1,4-dioxane, rt, 86%; (d) 1 M TBAF in THF, DCM, 40 °C, 40%; (e) H_2, Pt/C, MeOH, rt.

Scheme 3. Synthesis of compounds 17 and 18[a]



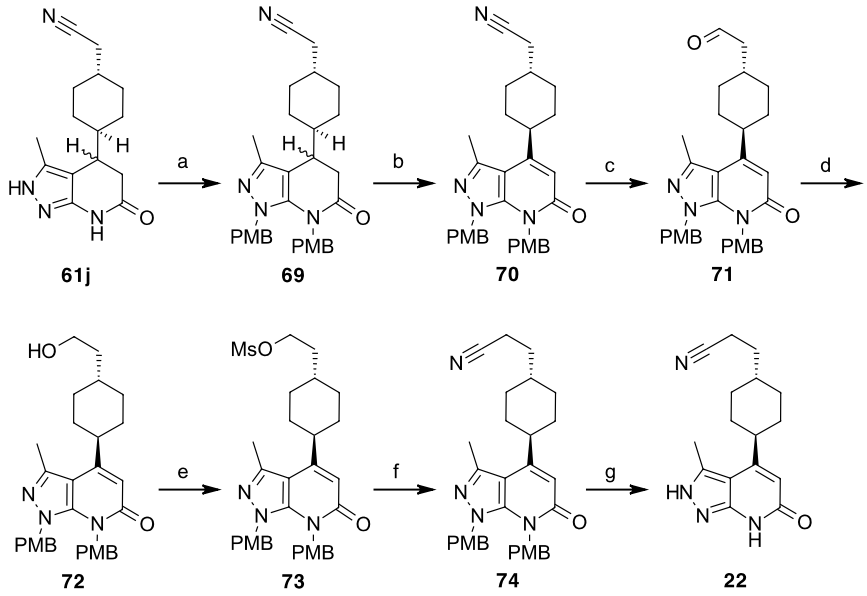
[a]Reagents and conditions: (a) $POCl_3$, DMF, 100 °C, then MnO_2, MeCN, rt, 15%; (b) 4.4 M NaOMe in MeOH, 120 °C, microwave heating, then $NaBH_4$, MeOH, rt, 81%; (c) 5 M HBr in AcOH, 40 °C, 23%; (d) 4.4 M NaOMe in MeOH, 120 °C, microwave heating, 99%; (e) PPh_3MeBr, 2.5 M *n*-BuLi, THF, 0 °C–rt, 91%; (f) 0.5 M 9-BBN, THF, then H_2O_2, NaOH, rt, 47%; (g) Pyridine hydrochloride, neat, 90 °C, 4.4%.

Scheme 4. Synthesis of compound 19[a]



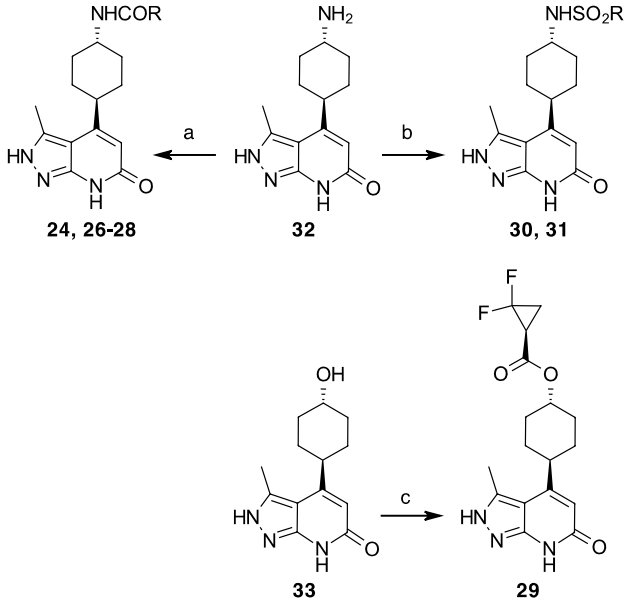
[a]Reagents and conditions: (a) MeOH, 60 °C, 47%; (b) NBS, DBU, MeCN, rt, 9.1%.

Scheme 5. Synthesis of compound 22[a]



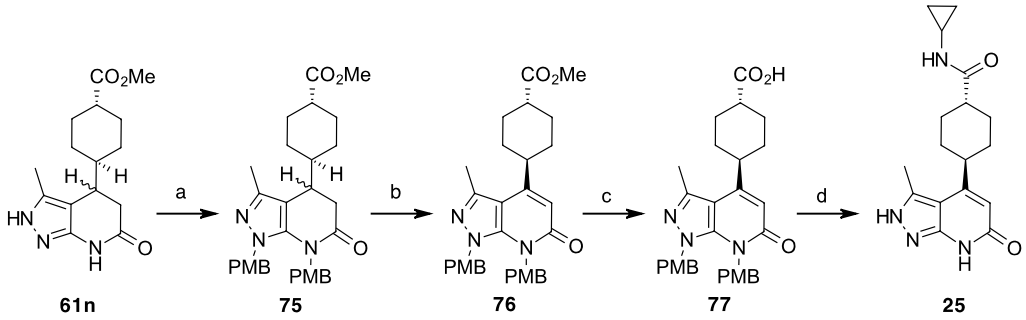
[a]Reagents and conditions: (a) Cs_2CO_3, PMBCl, DMF, rt, 53%; (b) DDQ, 1,4-dioxane, 100 °C, 50%; (c) 1.0 M DIBAL-H, toluene, 0 °C, 82%; (d) $NaBH_4$, THF, rt, 71%; (e) MsCl, Et_3N, DCM, rt, 69%; (f) NaCN, DMF, 90 °C, 66%; (g) TFA, reflux, 25%.

Scheme 6. Synthesis of compounds 24 and 26-31[a]



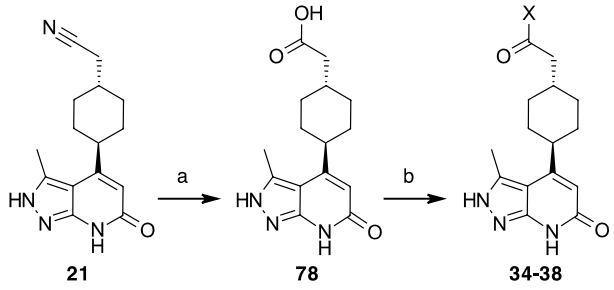
[a]Reagents and conditions: (a) RCO_2H, HATU, DIPEA, DMF, rt, library format; (b) RSO_2Cl, DIPEA, DMSO, rt, library format; (c) (1*S*)-2,2-difluorocyclopropanecarboxylic acid, HATU, DMAP, DMF, rt, 13%.

Scheme 7. Synthesis of compound 25[a]



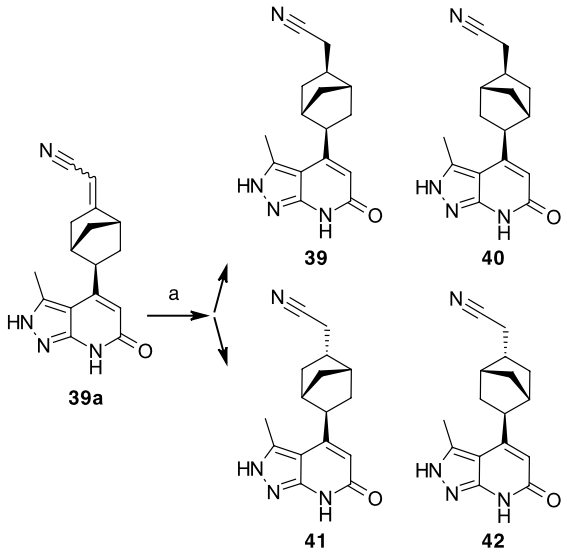
[a]Reagents and conditions: (a) Cs_2CO_3, PMBCl, DMF, rt, 92%; (b) DDQ, 1,4-dioxane, 100 °C, 87%; (c) LiOH, H_2O, THF, 40 °C, 50%; (d) cyclopropylamine, HATU, DIPEA, DMF, rt, then TFA, 90 °C, 9%.

Scheme 8. Synthesis of compounds 34-38[a]



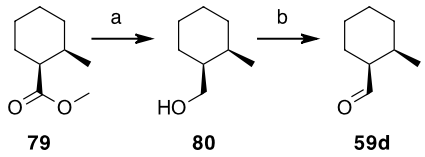
[a]Reagents and conditions: (a) Conc. HCl, 1,4-dioxane, 100 °C, 83%; (b) Amine, HATU, DIPEA, DMF, rt, library format.

Scheme 9. Synthesis of compounds 39-42[a]



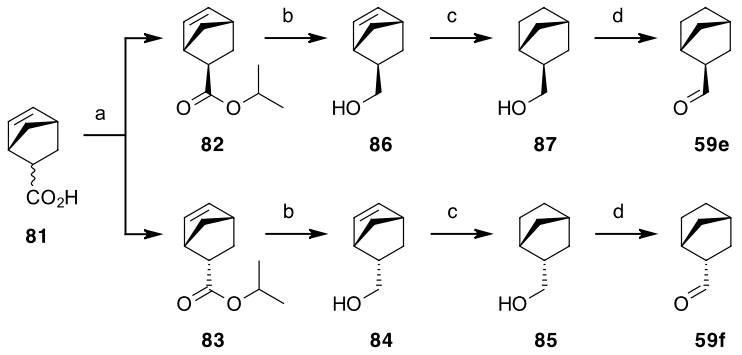
[a]Reagents and conditions: (a) H_2, Pt/C, MeOH, rt, then chiral SFC separation of stereoisomers. Compound **39a** is a mixture of *E* and *Z* isomers and the *E* and *Z* isomers are racemic. Compounds **39** and **40** are enantiomers of unknown absolute configurations, as are compounds **41** and **42**.

Scheme 10. Synthesis of aldehyde building block 59d[a]



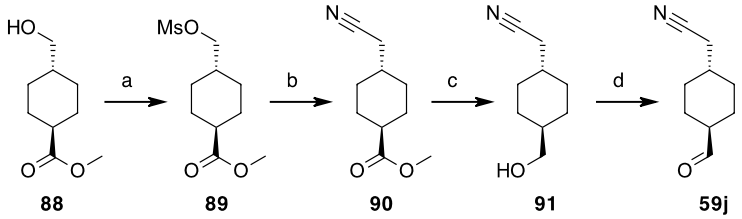
[a]Reagents and conditions: (a) $LiAlH_4$, THF, −30 °C, 83%; (b) Dess-Martin periodinane, $NaHCO_3$, DCM, rt, 30%.

Scheme 11. Synthesis of aldehyde building blocks 59e and 59f[a]



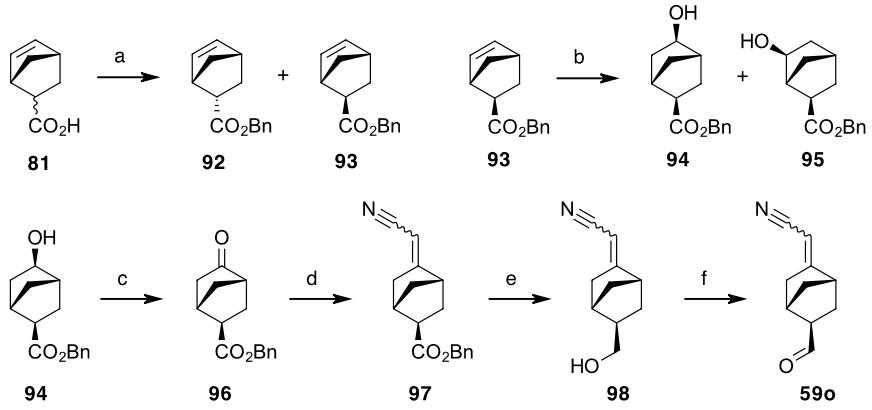
[a]Reagents and conditions: (a) 2-Propanol, EDCI, DMAP, 1,2-dichloroethane, rt, 14% (**82**), 55% (**83**); (b) $LiAlH_4$, THF, 40 °C; (c) H_2, Pd/C, EtOAc, rt, 96%; (d) $(COCl)_2$, DMSO, Et_3N, DCM, −78 °C, 68%.

Scheme 12. Synthesis of aldehyde building block 59j[a]



[a]Reagents and conditions: (a) MsCl, Et_3N, DCM, 0 °C, 99%; (b) KCN, DMSO, rt, 81%; (c) $LiBH_4$, THF, 40 °C, 84%; (d) $(COCl)_2$, DMSO, Et_3N, DCM, −78 °C, 75%.

Scheme 13. Synthesis of aldehyde building block 59o[a]



[a]Reagents and conditions: (a) Benzyl alcohol, DCC, DMAP, DCM, rt, 26% (**92**), 10% (**93**); (b) $BH_3 \cdot THF$, THF, −5 °C, then KH_2PO_4, H_2O_2, −5 °C, 25% (**94**), 25% (**95**); (c) Dess-Martin periodinane, DCM, rt, 54%; (d) $NCCH_2PO(OEt)_2$, *t*-BuOK, THF, rt, 68%; (e) $LiBH_4$, THF, rt, 87%; (f) Dess-Martin periodinane, DCM, rt, 94%.

EXPERIMENTAL

General biology experimental procedures are detailed in the supporting information. Most of the IC_{50} values reported in Tables 1 and 3-6 are geometric means of at least three independent experiments. The corresponding pIC_{50} values, their standard errors of the mean (SEM), and the number of experiments (N) are provided in the Molecular formula strings of the Supporting information.

The housing and husbandry of animals was undertaken in compliance with the European Committee Directive 2010/63/EU and performed under permit 2015-15-0201-00595 from the Danish authorities. Our animal welfare body inspects all experiments in our animal facility on a regular basis in order to secure implementation of the 3Rs in all experimental procedures.

Reagents and solvents were commercially available and were used without purification unless otherwise noted. Chromatographic purification was performed using a Grace REVELERIS system or a Teledyne ISCO Combi*Flash* Rf system with pre-packed silica gel cartridges, or manually using silica gel 60. Mass spectra of intermediates were recorded using a Shimadzu LCMS2020 system equipped with a LCMS2020 mass spectrometer operating in positive electrospray ionization mode. A Chromolith SpeedROD RP-18e column (50 × 4.6 mm, 2 µm particle size) at 40 °C was used (mobile phase: A = $CH_3CN/H_2O/HCO_2H$ = 10/90/0.05 and B = $CH_3CN/H_2O/HCO_2H$ = 90/10/0.05; flow rate: 3.0 mL/min; gradient: 0.8 min @ 10% B, 2.7 min gradient (10-95% B), then 0.8min @ 95% B). Alternatively, UPLC-MS analyses of intermediates were performed using a Waters Acquity UPLC system with a 2.1 × 50 mm Acquity UPLC HSS T3 1.8 µm column and an Acquity SQ Detector operated in positive ionization electrospray mode. The mobile phase consisted of 0.1% formic acid in an aqueous 10 mM ammonium acetate solution for buffer A and 0.1% formic acid in acetonitrile for buffer B. A binary gradient (A:B 95:5→5:95) over 1.4 min was used with a flow rate of 1.2 mL/min and the column temperature was 60 °C. Purities and high resolution mass spectra of all compounds tested in biological assays were determined using a Waters Acquity UPLC system with a 2.1 × 50 mm Acquity UPLC HSS T3 1.8 µm column, a Waters PDA detector, and a Waters XEVO G2-XS QTof or a Waters LCT Premier XE mass spectrometer. The same buffers A and B as above were used, but with a slower gradient (A:B 99:1→1:99 over 4.8 min; 0.7 mL/min; column temp. 30 °C). All compounds tested in biological assays were ≥95% pure by integration of peaks in the UV total absorbance chromatogram trace (240-400 nm) except compounds **17**, **37**, and **41** that were 94% pure, and compound **34** that was 81% pure. ^{1}H NMR spectra were recorded on Bruker instruments at 300, 400, or 600 MHz with tetramethylsilane as reference.

*Attempted synthesis of 4-(difluoromethyl)-3-methyl-2,7-dihydropyrazolo[3,4-b]pyridin-6-one (**1**).*[23-25] A solution of ethyl 4,4-difluoro-3-oxo-butanoate (171 mg, 1.03 mmol) and 3-methyl-1*H*-pyrazol-5-amine **43c** (100 mg, 1.03 mmol) in acetic acid (1 mL) was heated in a microwave synthesizer at 135 °C for 30 min. The reaction mixture partially solidified. Volatiles were evaporated and the residue was diluted with water (5 mL). The solid was collected by filtration and washed with water. It was then dried *in vacuo* overnight and recrystallized from methanol to afford 7-(difluoromethyl)-2-methyl-4*H*-pyrazolo[1,5-*a*]pyrimidin-5-one **50** (48 mg, 23%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.94 (br s, 1H), 7.03 (t, *J* = 53.5 Hz, 1H), 6.10 (s, 1H), 6.00 (s, 1H), 2.32 (s, 3H).

*4-(Difluoromethyl)-3-methyl-2,7-dihydropyrazolo[3,4-b]pyridin-6-one (**1**).*[26] To a solution of 2-*tert*-butyl-5-methyl-pyrazol-3-amine **51** (153 mg, 1.00 mmol) in acetic acid (1 mL) was added ethyl 4,4-difluoro-3-oxo-butanoate **49** (332 mg, 2.00 mmol) under argon. The resulting pale yellow solution was stirred at 110 °C for 17 h, cooled to rt, and volatiles were evaporated. To the residue was added sat. $NaHCO_3$ solution and the mixture was extracted twice with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and concentrated. The yellow solid residue was dried under high vacuum to afford 1-*tert*-butyl-4-(difluoromethyl)-3-methyl-7*H*-pyrazolo[3,4-*b*]pyridin-6-one **55** (263 mg, 98%) that was used without purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.81 (br s, 1H), 6.74 (t, *J* = 55.0 Hz, 1H), 6.51 (s, 1H), 2.48 (s, 3H), 1.73 (s, 9H).

A solution of 1-*tert*-butyl-4-(difluoromethyl)-3-methyl-7*H*-pyrazolo[3,4-*b*]pyridin-6-one **55** (200 mg, 0.744 mmol) in TFA (2 mL) was heated at 70 °C for 19 h. After cooling, volatiles were evaporated and sat. $NaHCO_3$ solution was added to the residue. The mixture was extracted with EtOAc (2 × 30 mL) and the combined organic layers were washed with brine and dried over Na_2SO_4. Upon concentration to a volume of ~3 mL a white solid precipitated. This was collected by filtration, washed with a small amount of EtOAc, and dried to afford 4-(difluoromethyl)-3-methyl-2,7-dihydropyrazolo[3,4-*b*]pyridin-6-one **1** (55.3 mg, 35%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.05 (br s, 1H), 11.88 (br s, 1H), 7.11 (t, *J* = 54.2 Hz, 1H), 6.31 (s, 1H), 2.43 (s, 3H). UPLC t_R = 1.65 min, Purity UV_{254} >99%. HRMS (ESI) m/z: $[M + H]^+$ Calcd for $C_8H_8F_2N_3O$ 200.0630; Found 200.0638.

*4-(Difluoromethyl)-2,7-dihydropyrazolo[3,4-b]pyridin-6-one (**3**).* Obtained as a white solid (86% in step 1, 16% in step 2) from 2-*tert*-butylpyrazol-3-amine **52** (400 mg, 2.9 mmol) and ethyl 4,4-difluoro-3-oxo-butanoate **49** (760 mg, 4.6 mmol) following the experimental procedure described for **1**. ^{1}H NMR (600 MHz, DMSO-d_6) δ 13.34 (br s, 1H), 12.01 (br s, 1H), 8.11 (s, 1H), 7.07 (t, *J* = 54.6 Hz, 1H), 6.34 (s, 1H). UPLC t_R = 1.53 min, Purity UV_{254} 98%. HRMS (ESI) m/z: $[M + H]^+$ Calcd for $C_7H_6F_2N_3O$ 186.0479; Found 186.0461.

*3-Methyl-4-phenyl-2,7-dihydropyrazolo[3,4-b]pyridin-6-one (**6**).* Obtained as a white solid (47% in step 1, 68% in step 2) from 2-*tert*-butyl-5-methyl-pyrazol-3-amine **51** (400 mg, 2.61 mmol) and methyl 3-oxo-3-phenyl-propanoate **53** (1.01 g, 5.25 mmol) following the experimental procedure described for **1**. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.87 (br s, 1H), 11.59 (br s, 1H), 7.52 – 7.45 (m, 5H), 5.86 (s, 1H), 2.00 (s, 3H). UPLC t_R = 1.83 min, Purity UV_{254} >99%. HRMS (ESI) m/z: $[M + H]^+$ Calcd for $C_{13}H_{12}N_3O$ 226.0975; Found 226.0961.

*4-Cyclohexyl-2,7-dihydropyrazolo[3,4-b]pyridin-6-one (**14**).* Obtained as a white solid (12% in step 1, 18% in step 2) from 2-*tert*-butylpyrazol-3-amine **52** (305 mg, 2.19 mmol) and 3-cyclohexyl-3-oxo-propanoate **54** (645 mg, 3.50 mmol) following the experimental procedure described for **1**. The product was purified by HPLC. ^{1}H NMR (600 MHz, DMSO-d_6) δ 13.03 (br s, 1H), 11.42 (br s, 1H), 8.19 (s, 1H), 5.86 (s, 1H), 2.68 – 2.58 (m, 1H), 1.88 – 1.76 (m, 4H), 1.74 – 1.68 (m, 1H), 1.49 – 1.36 (m, 4H), 1.33 – 1.22 (m, 1H). UPLC t_R = 1.89 min, Purity UV_{254} > 99%. HRMS (ESI) m/z: $[M + H]^+$ Calcd for $C_{12}H_{16}N_3O$ 218.1288; Found 218.1287.

*4-Cyclohexyl-3-methyl-2,7-dihydropyrazolo[3,4-b]pyridin-6-one (**7**).* A 25 mL round-bottom flask was charged with Meldrum's acid **60** (144 mg, 1.00 mmol) and ethanol (3 mL) under argon. To the white suspension was added cyclohexanecarbaldehyde **59a** (0.121 mL, 1.00 mmol). The

resulting suspension was stirred for 5 min at rt, 3-methyl-1*H*-pyrazol-5-amine **43c** (97.1 mg, 1.00 mmol) was added, and the mixture was heated to reflux. Initially, a pale yellow solution was formed. After 2.5 h at reflux, the resulting pale yellow suspension was cooled to rt. The solid was collected by filtration and was washed with ethanol (2 × 0.4 mL). The filter cake was dried under high vacuum (freeze dryer) overnight to afford 4-cyclohexyl-3-methyl-2,4,5,7-tetrahydropyrazolo[3,4-*b*]pyridin-6-one **61a** (147 mg, 63%) as a white solid.

To a suspension of 4-cyclohexyl-3-methyl-2,4,5,7-tetrahydropyrazolo[3,4-*b*]pyridin-6-one **61a** (35.0 mg, 0.150 mmol) and 1,4-dioxane (0.35 mL) was added DDQ (51.1 mg, 0.225 mmol). The mixture was heated at 100 °C for 2 h and was then cooled to rt over 2 h. Volatiles were removed by evaporation. To the residue was added sat. aq. $NaHCO_3$ solution (2 mL) and the mixture was extracted with DCM/MeOH 9:1 (5 × 3 mL). Because some solid remained in the water phase, the mixture was filtered. The organic phases from the extraction and the filter cake were combined, diluted with DCM/MeOH 7:3 (50 mL) and dried over Na_2SO_4. Filtration and evaporation afforded a crude product which was suspended in ethanol (0.5 mL). The solid was collected by filtration and the filter cake was washed with ethanol (4 × 0.15 mL). The filter cake was then dried under high vacuum (freeze dryer) overnight to afford 4-cyclohexyl-3-methyl-2,7-dihydropyrazolo[3,4-*b*]pyridin-6-one **7** (20.9 mg, 57%) as a pale yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.72 (br s, 1H), 11.36 (br s, 1H), 5.79 (s, 1H), 2.82 – 2.67 (m, 1H), 2.46 (s, 3H), 1.93 – 1.77 (m, 4H), 1.73 (d, *J* = 13.0 Hz, 1H), 1.48 – 1.16 (m, 5H). UPLC t_R = 1.95 min, Purity UV_{254} 96%. HRMS (ESI) m/z: $[M + H]^+$ Calcd for $C_{13}H_{18}N_3O$ 232.1444; Found 232.1469.

*4-Cyclopentyl-3-methyl-2,7-dihydropyrazolo[3,4-b]pyridin-6-one (**8**).* To a solution of cyclopentanecarbaldehyde **59b** (150 mg, 1.53 mmol) in ethanol (2 mL) was added Meldrum's acid **60** (220 mg, 1.53 mmol) and the mixture was stirred for 15 min at rt. Another portion of ethanol (3 mL) was added and the mixture was stirred for a further 10 min at rt. To the reaction mixture was added 3-methyl-1*H*-pyrazol-5-amine **43c** (148 mg, 1.53 mmol) and the mixture was heated at 70 °C for 1.5 hours during which time a precipitate formed. The mixture was cooled to rt and the solid was collected by filtration and dried under high vacuum (freeze dryer) to afford 4-cyclopentyl-3-methyl-2,4,5,7-tetrahydropyrazolo[3,4-*b*]pyridin-6-one **61b** (239 mg, 71%) as a white solid.

To a suspension of 4-cyclopentyl-3-methyl-2,4,5,7-tetrahydropyrazolo[3,4-*b*]pyridin-6-one **61b** (25 mg, 0.11 mmol) in 1,4-dioxane (2 mL) was added DDQ (44 mg, 0.19 mmol) and the mixture was heated at 100 °C for 25 min. After cooling to rt, the formed precipitate was collected by filtration, dissolved in DMSO and purified by HPLC (acidic method) to afford 4-cyclopentyl-3-methyl-2,7-dihydropyrazolo[3,4-*b*]pyridin-6-one **8** (7.8 mg, 32%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.69 (br s, 1H), 11.35 (br s, 1H), 5.84 (s, 1H), 3.28 (p, *J* = 7.7 Hz, 1H), 2.49 (s, 3H), 2.03 – 1.92 (m, 2H), 1.78 – 1.53 (m, 6H). UPLC t_R = 1.87 min, Purity UV_{254} >99%. HRMS (ESI) m/z: $[M + H]^+$ Calcd for $C_{12}H_{16}N_3O$ 218.1288; Found 218.1347.

*4-(Cyclobutylmethyl)-3-methyl-2,7-dihydropyrazolo[3,4-b]pyridin-6-one (**9**).* To a solution of cyclobutaneacetaldehyde **59c** (49 mg, 0.50 mmol) in pyridine (1 mL) was added piperidine (4.9 µL, 0.050 mmol) and Meldrum's acid **60** (72 mg, 0.50 mmol). The mixture was stirred at rt for 30 min. To the reaction mixture was added ethanol (1 mL) and 3-methyl-1*H*-pyrazol-5-amine **43c** (48 mg, 0.50 mmol) and the mixture was heated at 70 °C for 1 hour. Volatiles were evaporated and the residue was purified by chromatography (ISCO CombiFlash, 12 g silica gel column, heptane:EtOAc 100:0 → 0:100) to afford 4-(cyclobutylmethyl)-3-methyl-2,4,5,7-tetrahydropyrazolo[3,4-*b*]pyridin-6-one **61c** (25 mg, 23%) as a solid.

To a suspension of 4-(cyclobutylmethyl)-3-methyl-2,4,5,7-tetrahydropyrazolo[3,4-*b*]pyridin-6-one **61c** (25 mg, 0.11 mmol) in 1,4-dioxane (1 mL) was added DDQ (44 mg, 0.19 mmol) and the mixture was heated at 100 °C for 20 min. After cooling to rt, the precipitated solid was collected by filtration, dissolved in DMSO and purified by HPLC to afford 4-(cyclobutylmethyl)-3-methyl-2,7-dihydropyrazolo[3,4-*b*]pyridin-6-one **9** (3.0 mg, 12%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.70 (br s, 1H), 11.30 (br s, 1H), 5.72 (s, 1H), 2.77 (d, *J* = 7.3 Hz, 2H), 2.64 – 2.55 (m, 1H), 2.45 (s, 3H), 2.09 – 2.01 (m, 2H), 1.89 – 1.79 (m, 2H), 1.76 – 1.68 (m, 2H). UPLC t_R = 1.89 min, Purity UV_{254} >99%. HRMS (ESI) m/z: $[M + H]^+$ Calcd for $C_{12}H_{16}N_3O$ 218.1288; Found 218.1308.

3-Methyl-4-[(1S,2R*)-2-methylcyclohexyl]-2,7-dihydropyrazolo[3,4-b]pyridin-6-one (**10**).* To a solution of (1*S**,2*R**)-2-methylcyclohexanecarbaldehyde **59d** (0.600 g, 4.75 mmol) in ethanol (10 mL) was added Meldrum's acid **60** (0.685 g, 4.75 mmol). The reaction mixture was stirred at rt for 15 min followed by the addition of 3-methyl-1*H*-pyrazol-5-amine **43c** (0.369 g, 3.80 mmol) and the resulting mixture was stirred at 80 °C for 3 h. After cooling to rt the mixture was concentrated under reduced pressure. The residue was washed with diethyl ether. The solid thus obtained was dried under vacuum to afford 3-methyl-4-[(1*S**,2*R**)-2-methylcyclohexyl]-2,4,5,7-tetrahydropyrazolo[3,4-*b*]pyridin-6-one **61d** (0.250 g, 27%) as an off-white solid (mixture of diastereomers). ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 11.69 (s, 1H), 10.06 (s, 1H), 2.54 (m, 1H), 2.25 (m, 2H), 2.05 (s, 3H) 1.75 – 1.45 (m, 6H), 1.45 – 1.20 (m, 2H), 0.9 (m, 3H).

To a solution of 3-methyl-4-[(1*S**,2*R**)-2-methylcyclohexyl]-2,4,5,7-tetrahydropyrazolo[3,4-*b*]pyridin-6-one **61d** (0.250 g, 1.01 mmol) in 1,4-dioxane (10 mL) was added DDQ (0.456 g, 1.52 mmol). The reaction mixture was stirred at 100 °C for 3h. After cooling, the mixture was concentrated under reduced pressure. The residue was purified by HPLC to afford 3-methyl-4-[(1*S**,2*R**)-2-methylcyclohexyl]-2,7-dihydropyrazolo[3,4-*b*]pyridin-6-one **10** (0.040 g, 16%) as an off-white solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.72 (br s, 1H), 11.30 (br s, 1H), 5.65 (s, 1H), 3.01 (br d, *J* = 11.5 Hz, 1H), 2.15 – 2.04 (m, 1H), 1.87 – 1.56 (m, 4H), 1.54 – 1.31 (m, 4H), 0.72 (d, *J* = 7.1 Hz, 3H). UPLC t_R = 2.02 min, Purity UV_{254} >99%. HRMS (ESI) m/z: $[M + H]^+$ Calcd for $C_{14}H_{19}N_3O$ 246.1601; Found 246.1622.

3-Methyl-4-[(1R,2R*,4S*)-norbornan-2-yl]-2,7-dihydropyrazolo[3,4-b]pyridin-6-one (**11**).* To a solution of norbornane-2-*exo*-carbaldehyde **59e** (137 mg, 1.10 mmol) in ethanol (2 mL) was added Meldrum's acid **60** (159 mg, 1.10 mmol). The reaction mixture was stirred at rt for 5 min followed by the addition of 3-methyl-1*H*-pyrazol-5-amine **43c** (107 mg, 1.10 mmol) and the resulting mixture was stirred at reflux for 4 h. After cooling to rt the mixture was concentrated under reduced pressure. The residue was stirred with EtOAc (3 mL) for 30 min. The solid thus obtained was collected by filtration, washed with EtOAc (2 × 0.5 mL) and dried under vacuum to afford 3-methyl-4-[(1*R**,2*R**,4*S**)-norbornan-2-yl]-2,4,5,7-tetrahydropyrazolo[3,4-*b*]pyridin-6-one **61e** (180 mg, 60%) as a white solid (mixture of diastereomers).

To a suspension of 3-methyl-4-[(1*R**,2*R**,4*S**)-norbornan-2-yl]-2,4,5,7-tetrahydropyrazolo[3,4-*b*]pyridin-6-one **61e** (180 mg, 0.66 mmol) in 1,4-dioxane (3 mL) was added DDQ (255 mg, 1.12 mmol) and the mixture was heated at 100 °C for 2 h. After cooling to rt the mixture was concentrated under reduced pressure. The residue was suspended in sat. $NaHCO_3$ solution (20 mL) and the mixture was extracted with DCM:MeOH 9:1 (3 × 40 mL). The combined organic phases were washed with brine (20 mL), dried over Na_2SO_4, and volatiles were evaporated. The residue was purified by column chromatography (silica gel column, DCM:MeOH 96:4 → 94:6) to afford 3-Methyl-4-[(1*R**,2*R**,4*S**)-norbornan-2-yl]-2,7-dihydropyrazolo[3,4-*b*]pyridin-6-one **11** (39.2 mg, 22%) as a yellow solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.71 (br s, 1H), 11.30 (br s, 1H), 5.76 (br s, 1H), 2.92 (br s, 1H), 2.49 (s, 3H), 2.32 (br s, 1H), 2.29 (br d, *J* = 4.1 Hz, 1H), 1.67 – 1.50 (m, 4H), 1.45 – 1.37 (m, 2H), 1.32 – 1.26 (m, 1H), 1.20 – 1.14 (m, 1H). UPLC t_R = 1.97 min, Purity UV_{254} >99%. HRMS (ESI) m/z: $[M + H]^+$ Calcd for $C_{14}H_{18}N_3O$ 244.1444; Found 244.1427.

3-Methyl-4-[(1R,2S*,4S*)-norbornan-2-yl]-2,7-dihydropyrazolo[3,4-b]pyridin-6-one (**12**).* To a solution of norbornane-2-*endo*-carbaldehyde **59f** (107 mg, 0862 mmol) in ethanol (1.5 mL) was added Meldrum's acid **60** (124 mg, 0.862 mmol). The reaction mixture was stirred at rt for 5 min followed by the addition of 3-methyl-1*H*-pyrazol-5-amine **43c** (83.7 mg, 0.862 mmol) and the resulting mixture was stirred at reflux for 4 h. The resulting pale yellow suspension was cooled in an ice bath. The solid was collected by filtration and was washed with ice cold ethanol (3 × 0.2 mL). Drying under high vacuum afforded [(1*R**,2*S**,4*S**)-norbornan-2-yl]-2,4,5,7-tetrahydropyrazolo[3,4-*b*]pyridin-6-one **61f** (88.5 mg, 35%) as a white solid (mixture of diastereomers).

To a suspension of 3-methyl-4-[(1*R**,2*S**,4*S**)-norbornan-2-yl]-2,4,5,7-tetrahydropyrazolo[3,4-*b*]pyridin-6-one **61f** (84.3 mg, 0.344 mmol) in 1,4-dioxane (1.4 mL) was added DDQ (117 mg, 0.515 mmol) and the mixture was heated at 100 °C for 2 h. After cooling to rt the mixture was concentrated under reduced pressure. The residue was suspended in sat. $NaHCO_3$ solution (10 mL) and the mixture was extracted with DCM:MeOH 9:1 (3 × 20 mL). The combined organic phases were washed with brine (10 mL), dried over Na_2SO_4, and volatiles were evaporated. The residue was purified by column chromatography (silica gel column, DCM:MeOH 96:4 → 95:5) to afford 3-methyl-4-[(1*R**,2*S**,4*S**)-norbornan-2-yl]-2,7-dihydropyrazolo[3,4-*b*]pyridin-6-one **12** (29.8 mg, 34%) as a yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.69 (br s, 1H), 11.34 (br s, 1H), 5.89 (s, 1H), 3.53 – 3.40 (m, 1H), 2.50 (s, 3H), 2.40 – 2.24 (m, 2H), 1.82 – 1.69 (m, 1H), 1.69 – 1.60 (m, 1H), 1.58 – 1.36 (m, 3H), 1.33 – 1.15 (m, 3H). UPLC t_R = 1.95 min, Purity UV_{254} 99%. HRMS (ESI) m/z: $[M + H]^+$ Calcd for $C_{14}H_{18}N_3O$ 244.1444; Found 244.1438.

*4-(2-Bicyclo[2.2.2]octanyl)-3-methyl-2,7-dihydropyrazolo[3,4-b]pyridin-6-one (**13**).* To a solution of bicyclo[2.2.2]octane-2-carbaldehyde **59g** (74.3 mg, 0.473 mmol) in ethanol (1.4 mL) was added Meldrum's acid **60** (68.2 mg, 0.473 mmol). The reaction mixture was stirred at rt for 5 min followed by the addition of 3-methyl-1*H*-pyrazol-5-amine **43c** (45.9 mg, 0.473 mmol) and the resulting mixture was stirred at reflux for 4 h. The resulting pale yellow suspension was cooled in an ice bath. The solid was collected by filtration and was washed with ice cold ethanol (2 × 0.3 mL). Drying under high vacuum afforded 4-(2-bicyclo[2.2.2]octanyl)-3-methyl-2,4,5,7-tetrahydropyrazolo[3,4-*b*]pyridin-6-one **61g** (63.3 mg, 49%) as a white solid (mixture of diastereomers).

To a suspension of 4-(2-bicyclo[2.2.2]octanyl)-3-methyl-2,4,5,7-tetrahydropyrazolo[3,4-*b*]pyridin-6-one **61g** (59.0 mg, 0.227 mmol) in 1,4-dioxane (1.0 mL) was added DDQ (77.5 mg, 0.341 mmol) and the mixture was heated at 100 °C for 1.5 h. After cooling to rt the mixture was concentrated under reduced pressure. The residue was dissolved in DCM:MeOH 95:5 (8 mL), washed with sat. $NaHCO_3$ solution (10 mL) and the aqueous layer was extracted with DCM:MeOH 95:5 (4 mL). The combined organic phases were dried over Na_2SO_4 and volatiles were evaporated. The residue was purified by column chromatography (silica gel column, DCM:MeOH 96:4 → 94:6) to afford 4-(2-bicyclo[2.2.2]octanyl)-3-methyl-2,7-dihydropyrazolo[3,4-b]pyridin-6-one **13** (25.8 mg, 42%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.63 (br s, 1H), 11.37 (br s, 1H), 5.97 (s, 1H), 3.30 – 3.19 (m, 1H), 2.45 (s, 3H), 1.88 – 1.39 (m, 11H), 1.37 – 1.22 (m, 1H). UPLC t_R = 2.03 min, Purity UV_{254} >99%. HRMS (ESI) m/z: $[M + H]^+$ Calcd for $C_{15}H_{20}N_3O$ 258.1601; Found 258.1567.

*4-Cyclohexyl-3-ethyl-2,7-dihydropyrazolo[3,4-b]pyridin-6-one (**15**).* To a solution of cyclohexanecarbaldehyde **59a** (63 mg, 0.56 mmol) in ethanol (2.7 mL) was added Meldrum's acid **60** (81 mg, 0.56 mmol). The reaction mixture was stirred at rt for 5 min followed by the addition of 3-ethyl-1*H*-pyrazol-5-amine **43d** (62 mg, 0.56 mmol) and the resulting mixture was stirred at 60 °C overnight. The reaction mixture was cooled, concentrated to half its volume, and ether (2 mL) was added to afford a white precipitate. The white solid was collected by filtration and was washed with ether. Drying under high vacuum afforded 4-cyclohexyl-3-ethyl-2,4,5,7-tetrahydropyrazolo[3,4-*b*]pyridin-6-one **61h** (120 mg, 86%) as a white solid.

To a suspension of 4-cyclohexyl-3-ethyl-2,4,5,7-tetrahydropyrazolo[3,4-*b*]pyridin-6-one **61h** (20 mg, 0.081 mmol) in 1,4-dioxane (1.0 mL) was added DDQ (128 mg, 0.562 mmol) and the mixture was heated at 100 °C for 15 min. After cooling to rt the resulting white precipitate was collected by filtration and washed with methanol. The

crude product was purified by HPLC (acidic method) to afford 4-cyclohexyl-3-ethyl-2,7-dihydropyrazolo[3,4-*b*]pyridin-6-one **15** (4.1 mg, 21%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.73 (br s, 1H), 11.33 (br s, 1H), 5.82 (s, 1H), 2.85 (q, *J* = 7.6 Hz, 2H), 2.72 (s, 1H), 1.89 – 1.77 (m, 4H), 1.76 – 1.69 (m, 1H), 1.47 – 1.32 (m, 4H), 1.27 (t, *J* = 7.6 Hz, 3H), 1.26 – 1.21 (m, 1H). UPLC t_R = 2.02 min, Purity UV_{254} >99%. HRMS (ESI) m/z: $[M + H]^+$ Calcd for $C_{14}H_{20}N_3O$ 246.1601; Found 246.1614.

*4-Cyclohexyl-3-cyclopropyl-2,7-dihydropyrazolo[3,4-b]pyridin-6-one (**16**).* To a solution of cyclohexanecarbaldehyde **59a** (100 mg, 0.892 mmol) in ethanol (2.7 mL) was added Meldrum's acid **60** (129 mg, 0.892 mmol). The reaction mixture was stirred at rt for 5 min followed by the addition of 3-cyclopropyl-1*H*-pyrazol-5-amine **43e** (110 mg, 0.892 mmol) and the resulting mixture was stirred at 60 °C overnight. The reaction mixture was cooled, concentrated to half its volume, and ether (5 mL) was added to afford a white precipitate. The white solid was collected by filtration and was washed with ether. Drying under high vacuum afforded 4-cyclohexyl-3-cyclopropyl-2,4,5,7-tetrahydropyrazolo[3,4-*b*]pyridin-6-one **61i** (144 mg, 62%) as a white solid.

To a suspension of 4-cyclohexyl-3-cyclopropyl-2,4,5,7-tetrahydropyrazolo[3,4-*b*]pyridin-6-one **61i** (20 mg, 0.077 mmol) in 1,4-dioxane (1.0 mL) was added DDQ (202 mg, 0.892 mmol) and the mixture was heated at 100 °C for 15 min. After cooling to rt the resulting white precipitate was collected by filtration and washed with methanol. Vacuum drying afforded 4-cyclohexyl-3-cyclopropyl-2,7-dihydro-pyrazolo[3,4-*b*]pyridin-6-one **16** (4.4 mg, 22%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.47 (br s, 1H), 11.36 (br s, 1H), 5.83 (s, 1H), 3.04 (br t, 1H), 2.15 – 2.07 (m, 1H), 1.98 – 1.90 (m, 2H), 1.86 – 1.78 (m, 2H), 1.76 – 1.69 (m, 1H), 1.46 – 1.31 (m, 4H), 1.30 – 1.21 (m, 1H), 1.02 – 0.95 (m, 2H), 0.93 – 0.88 (m, 2H). UPLC t_R = 2.05 min, Purity UV_{254} >99%. HRMS (ESI) m/z: $[M + H]^+$ Calcd for $C_{15}H_{20}N_3O$ 258.1601; Found 258.1624.

*4-Cyclohexyl-5-(hydroxymethyl)-3-methyl-2,7-dihydropyrazolo[3,4-b]pyridin-6-one (**17**).* A mixture of 6-chloro-4-cyclohexyl-3-methyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbaldehyde **62** (0.15 g, 0.54 mmol) and 4.4 M NaOMe in MeOH (1.0 mL, 4.4 mmol) in MeOH (2 mL) was heated in the microwave at 120 °C for 40 min. After cooling to rt, $NaBH_4$ (10 mg, 0.27 mmol) was added and stirred until the yellow color disappeared and a clear solution was obtained (ca. 30 min). The reaction was quenched by adding AcOH, volatiles were evaporated, the residue was triturated with water and the solid was collected by filtration and dried *in vacuo* to afford (4-cyclohexyl-6-methoxy-3-methyl-1*H*-pyrazolo[3,4-*b*]pyridin-5-yl)methanol **64** (120 mg, 81%) as an off-white solid that was used directly in the following step.

A mixture of (4-cyclohexyl-6-methoxy-3-methyl-1*H*-pyrazolo[3,4-*b*]pyridin-5-yl)methanol **64** (22.0 mg, 0.0799 mmol) and 5 M HBr in AcOH (0.50 mL, 2.5 mmol) was stirred at 40 °C for 30 min. After cooling to rt, water (0.50 mL) was added, the mixture was purified by HPLC and pure fractions combined and freeze dried to afford 4-cyclohexyl-5-(hydroxymethyl)-3-methyl-2,7-dihydropyrazolo[3,4-*b*]pyridin-6-one **17** (4.9 mg, 23%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.70 (br s, 1H), 11.46 (br s, 1H), 4.49 (s, 2H), 3.08 (br s, 1H), 2.55 (br s, 3H), 1.91 – 1.56 (m, 7H), 1.47 – 1.21 (m, 3H). Very broad peaks, probably due to slow interconversion of tautomers. UPLC t_R = 1.86 min, Purity UV_{254} 94%. HRMS (ESI) m/z: $[M + H]^+$ Calcd for $C_{14}H_{20}N_3O_2$ 262.1550; Found 262.1529.

*4-Cyclohexyl-5-(2-hydroxyethyl)-3-methyl-2,7-dihydropyrazolo[3,4-b]pyridin-6-one (**18**).* A mixture of 6-chloro-4-cyclohexyl-3-methyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbaldehyde **62** (0.30 g, 1.1 mmol), 4.4 M NaOMe solution in MeOH (2.0 mL, 8.8 mmol) and MeOH (10 mL) was heated in the microwave at 120 °C for 40 min. After cooling to rt, the reaction was quenched with AcOH (1 mL) and volatiles were evaporated. The residue was dissolved in ether (25 mL) and washed with brine (10 mL). The organic layer was dried over Na_2SO_4 and evaporated to dryness to afford 4-cyclohexyl-6-methoxy-3-methyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbaldehyde **63** (298 mg, 99%) which was used without further purification.

A 2.5 M solution of *n*-BuLi in heptanes (0.29 mL, 0.73 mmol) was added slowly to a suspension of methyl(triphenyl)phosphonium bromide (261 mg, 0.732 mmol) in dry THF (10 mL) at 0 °C under argon. The resulting orange solution was stirred for 30 min at 0 °C. A solution of 4-cyclohexyl-6-methoxy-3-methyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbaldehyde **63** (100 mg, 0.366 mmol) in dry THF (5 mL) was added and the mixture was stirred for 6 h at rt. The reaction was quenched with AcOH (0.5 mL), diluted with water (10 mL) and extracted with DCM (2 × 10 mL). The combined organic layers were dried over Na_2SO_4 and evaporated. The residue was purified by chromatography (ISCO CombiFlash, silica gel column, heptane:EtOAc) to afford 4-cyclohexyl-6-methoxy-3-methyl-5-vinyl-1*H*-pyrazolo[3,4-*b*]pyridine **65** (90 mg, 91%) as a clear oil.

A 0.50 M solution of 9-BBN in THF (1.3 mL, 0.65 mmol) was added to a solution of 4-cyclohexyl-6-methoxy-3-methyl-5-vinyl-1*H*-pyrazolo[3,4-*b*]pyridine **65** (90.0 mg, 0.332 mmol) in dry THF (4 mL) and the mixture was stirred at rt for 3 h. A solution of 50% H_2O_2 (0.123 mL, 1.99 mmol) in 4M NaOH (1 mL) was added carefully and the resulting mixture was evaporated. The residue was purified by HPLC to afford 2-(4-cyclohexyl-6-methoxy-3-methyl-1*H*-pyrazolo[3,4-*b*]pyridin-5-yl)ethanol **66** (45 mg, 47%) as a clear oil.

A mixture of pyridine hydrochloride (500 mg) and 2-(4-cyclohexyl-6-methoxy-3-methyl-1*H*-pyrazolo[3,4-*b*]pyridin-5-yl)ethanol **66** (45 mg, 0.16 mmol) was stirred at 90 °C for 45 min. Water (0.5 mL) was added, the mixture was purified by HPLC and pure fractions combined and freeze dried to afford 4-cyclohexyl-5-(2-hydroxyethyl)-3-methyl-2,7-dihydropyrazolo[3,4-*b*]pyridin-6-one **18** (1.9 mg, 4.4%) as a white solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.84 (br s, 1H), 11.73 (br d, 1H), 5.62 (br s, 0.4H), 5.44 (d, *J* = 10.5 Hz, 0.6H), 5.26 (br s, 0.4H), 5.05 (br s, 0.6H), 3.50 (br s, 0.4H), 3.07 (br s, 0.6H), 2.66 (br s, 1H), 2.53 (br s, 2H), 1.92 – 1.23 (m, 13H). Two sets of broad peaks, *ca*. 4:6, probably due to two slowly interconverting tautomers. UPLC t_R = 1.97 min, Purity UV_{254} >99%. HRMS (ESI) m/z: $[M + H]^+$ Calcd for $C_{15}H_{22}N_3O_2$ 276.1706; Found 276.1700.

*4-Cyclohexyl-3-methyl-6-oxo-2,7-dihydropyrazolo[3,4-b]pyridine-5-carbonitrile (**19**)*. A mixture of 3-methyl-1*H*-pyrazol-5-amine **43c** (1.18 g, 12.2 mmol) and methyl (*Z*)-2-cyano-3-cyclohexyl-prop-2-enoate **67** (2.35 g, 12.2 mmol) in MeOH (15 mL) was stirred at 60 °C for 18 h. Volatiles were evaporated and the residue was purified by column chromatography (ISCO CombiFlash, silica gel column, DCM:MeOH) to afford 4-cyclohexyl-3-methyl-6-oxo-2,4,5,7-tetrahydropyrazolo[3,4-*b*]pyridine-5-carbonitrile **68** (1.47 g, 47%) as a pale orange solid (mixture of diastereomers).

To a solution of 4-cyclohexyl-3-methyl-6-oxo-2,4,5,7-tetrahydropyrazolo[3,4-*b*]pyridine-5-carbonitrile **68** (62.0 mg, 0.240 mmol) in MeCN (5 mL) was added NBS (42.7 mg, 0.240 mmol) at rt. The mixture was stirred for 30 min at rt after which DBU (72 µL, 0.48 mmol) was added and stirring was continued for an additional 10 min at rt. Volatiles were evaporated and the residue was purified by HPLC to afford 4-cyclohexyl-3-methyl-6-oxo-2,7-dihydropyrazolo[3,4-b]pyridine-5-carbonitrile **19** (5.6 mg, 9.1%) as a white solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 13.08 (br s, 1H), 12.51 (br s, 1H), 3.13 (br t, *J* = 12.6 Hz, 1H), 2.57 (s, 3H), 2.14 – 1.95 (m, 2H), 1.91 – 1.69 (m, 5H), 1.45 – 1.34 (m, 2H), 1.32 – 1.18 (m, 1H). UPLC t_R = 1.98 min, Purity UV_{254} >99%. HRMS (ESI) m/z: $[M + H]^+$ Calcd for $C_{14}H_{17}N_4O$ 257.1397; Found 257.1392.

*2-[trans-4-(3-Methyl-6-oxo-2,7-dihydropyrazolo[3,4-b]pyridin-4-yl)cyclohexyl]acetonitrile (**21**)*. To a solution of 2-(*trans*-4-formylcyclohexyl)acetonitrile **59j** (500 mg, 3.31 mmol) in ethanol (16.5 mL) was added Meldrum's acid **60** (477 mg, 3.31 mmol). The reaction mixture was stirred at rt for 5 min followed by the addition of 3-methyl-1*H*-pyrazol-5-amine **43c** (321 mg, 3.31 mmol) and the resulting mixture was stirred at 60 °C overnight. After cooling to rt the mixture was concentrated to half its volume under reduced pressure and ether (5 mL) was added. The solid thus obtained was collected by filtration, washed with ether and dried under vacuum to afford 2-[4-(3-methyl-6-oxo-2,4,5,7-tetrahydropyrazolo[3,4-*b*]pyridin-4-yl)cyclohexyl]acetonitrile **61j** (764 mg, 85%) which was used without purification.

To a suspension of 2-[4-(3-methyl-6-oxo-2,4,5,7-tetrahydropyrazolo[3,4-*b*]pyridin-4-yl)cyclohexyl]acetonitrile **61j** (100 mg, 0.367 mmol) in 1,4-dioxane (8 mL) was added DDQ (83.4 mg, 0.367 mmol) and the mixture was heated at 100 °C for 30 min. After cooling to rt the resulting white precipitate was collected by filtration and washed with 1,4-dioxane. The crude solid was purified using HPLC (acidic method) to afford 2-[*trans*-4-(3-methyl-6-oxo-2,7-dihydropyrazolo[3,4-*b*]pyridin-4-yl)cyclohexyl]acetonitrile **21** (57 mg, 57%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.73 (br s, 1H), 11.32 (br s, 1H), 5.81 (br s, 1H), 2.81 – 2.66 (m, 1H), 2.50 (d, *J* = 6.5 Hz, 2H), 2.47 (s, 3H), 1.94 – 1.84 (m, 4H), 1.76 – 1.65 (m, 1H), 1.49 – 1.38 (m, 2H), 1.32 – 1.19 (m, 2H). UPLC t_R = 1.80 min, Purity UV_{254} 97%. HRMS (ESI) m/z: $[M + H]^+$ Calcd for $C_{15}H_{19}N_4O$ 271.1553; Found 271.1574.

*3-[trans-4-(3-Methyl-6-oxo-2,7-dihydropyrazolo[3,4-b]pyridin-4-yl)cyclohexyl]propanenitrile (**22**)*. A solution of 3-[4-[1,7-*bis*[(4-methoxyphenyl)methyl]-3-methyl-6-oxo-pyrazolo[3,4-*b*]pyridin-4-yl]cyclohexyl]propanenitrile **74** (50 mg, 0.09 mmol) in TFA (4 mL) was prepared at 0 °C, and was then refluxed for 6 h. On completion, the reaction mixture was concentrated under reduced pressure. The crude product was diluted with MeOH and basified with Amberlyst A21 free base. The reaction mixture was filtered, the filtrate was concentrated under reduced pressure and the crude product was purified by HPLC to afford 3-[*trans*-4-(3-methyl-6-oxo-2,7-dihydropyrazolo[3,4-b]pyridin-4-yl)cyclohexyl]propanenitrile **22** (6.9 mg, 25%) as an off-white solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.73 (br s, 1H), 11.29 (br s, 1H), 5.78 (br s, 1H), 2.76 – 2.66 (br m, 1H), 2.54 (s, 2H), 2.46 (s, 3H), 1.92 – 1.82 (m, 4H), 1.53 (q, J = 7.2 Hz, 2H), 1.44 – 1.33 (m, 3H), 1.17 – 1.06 (m, 2H). UPLC t_R = 1.88 min, Purity UV_{254} >99%. HRMS (ESI) m/z: $[M + H]^+$ Calcd for $C_{16}H_{21}N_4O$ 285.1710; Found 285.1729.

*trans-4-(3-Methyl-6-oxo-2,7-dihydropyrazolo[3,4-b]pyridin-4-yl)cyclohexanecarbonitrile (**23**)*. To a solution of *trans*-4-formylcyclohexanecarbonitrile **59k**[33] (212 mg, 1.55 mmol) in ethanol (8 mL) was added Meldrum's acid **60** (223 mg, 1.55 mmol). The reaction mixture was stirred at rt for 5 min followed by the addition of 3-methyl-1*H*-pyrazol-5-amine **43c** (150 mg, 1.55 mmol) and the resulting mixture was stirred at 60 °C for 2 h. After cooling to rt, the mixture was concentrated to half its volume under reduced pressure and ether (10 mL) was added. The solid thus obtained was collected by filtration, washed with ether and dried to afford 4-(3-methyl-6-oxo-2,4,5,7-tetrahydropyrazolo[3,4-*b*]pyridin-4-yl)cyclohexanecarbonitrile **61k** (341 mg, 85%) which was used without purification.

To a suspension of 4-(3-methyl-6-oxo-2,4,5,7-tetrahydropyrazolo[3,4-*b*]pyridin-4-yl)cyclohexanecarbonitrile **61k** (341 mg, 1.32 mmol) in 1,4-dioxane (10 mL) was added DDQ (300 mg, 1.32 mmol) and the mixture was heated at 100 °C for 15 min. After cooling to rt the resulting white precipitate was collected by filtration and washed with 1,4-dioxane. It was triturated with MeOH to afford *trans*-4-(3-methyl-6-oxo-2,7-dihydropyrazolo[3,4-*b*]pyridin-4-yl)cyclohexanecarbonitrile as a light brown solid **23** (79 mg, 23%). A sample (7 mg) was further purified using HPLC (acidic method) to afford an analytically pure sample. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.80 (br s, 1H), 11.40 (br s, 1H), 5.75 (s, 1H), 2.87 – 2.78 (m, 1H), 2.77 (tt, *J* = 12.3, 3.7 Hz, 1H), 2.47 (s, 3H), 2.16 – 2.09 (m, 2H), 1.91 – 1.83 (m, 2H), 1.76 – 1.66 (m, 2H), 1.47 – 1.36 (m, 2H). UPLC t_R = 1.75 min, Purity UV_{254} >99%. HRMS (ESI) m/z: $[M + H]^+$ Calcd for $C_{14}H_{17}N_4O$ 257.1397; Found 257.1414.

General procedure for amide formation in library format. One amine, multiple acids. A stock solution of 4-(4-*trans*-aminocyclohexyl)-3-methyl-2,7-dihydropyrazolo[3,4-*b*]pyridin-6-one **32** (45 mg, 0.18 mmol) in DMF (3.6 mL) was prepared, and HATU (69 mg, 0.18 mmol) and DIPEA (94 µL, 0.54 mmol) were added. To each acid (20 µmol) in the library was added an aliquot of the stock solution (0.40 mL) and the resulting mixture was shaken at rt for 15 min. The reaction mixtures were purified by HPLC (acidic method) without prior work-up to afford the corresponding amides.

*N-[trans-4-(3-Methyl-6-oxo-2,7-dihydropyrazolo[3,4-b]pyridin-4-yl)cyclohexyl]cyclopropanecarboxamide (**24**)*. Prepared using the general procedure. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.75 (br s, 1H), 11.31 (br s, 1H), 7.95 (d, *J* = 7.8 Hz, 1H), 5.80 (br s, 1H), 3.66 – 3.55 (m, 1H), 2.78 – 2.66 (br m, 1H), 2.48 (s, 3H), 1.96 – 1.83 (m, 4H), 1.55 – 1.40 (m, 3H), 1.40 – 1.30 (m, 2H), 0.69 – 0.58 (m, 4H). UPLC t_R = 1.72 min, Purity UV_{254} 98%. HRMS (ESI) m/z: $[M + H]^+$ Calcd for $C_{17}H_{23}N_4O_2$ 315.1816; Found 315.1820.

*N-[trans-4-(3-Methyl-6-oxo-2,7-dihydropyrazolo[3,4-b]pyridin-4-yl)cyclohexyl]cyclopentanecarboxamide (**26**)*. Prepared using the general procedure. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.74 (br s, 1H), 11.31 (br s, 1H), 7.62 (d, *J* = 7.8 Hz, 1H), 5.80 (br s, 1H), 3.58 (tdt, *J* = 11.7, 8.0, 4.0 Hz, 1H), 2.80 – 2.63 (br m, 1H), 2.53 – 2.50 (m, 1H), 2.47 (s, 3H), 1.94 – 1.83 (m, 4H), 1.76 – 1.67 (m, 2H), 1.65 – 1.55 (m, 4H), 1.54 – 1.41 (m, 4H), 1.38 – 1.29 (m, 2H). UPLC t_R = 1.86 min, Purity UV_{254} >99%. HRMS (ESI) m/z: $[M + H]^+$ Calcd for $C_{19}H_{27}N_4O_2$ 343.2129; Found 343.2133.

*(1S)-2,2-Difluoro-N-[trans-4-(3-methyl-6-oxo-2,7-dihydropyrazolo[3,4-b]pyridin-4-yl)cyclohexyl]cyclopropanecarboxamide (**27**)*. Prepared using the general procedure. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.75 (br s, 1H), 11.31 (br s, 1H), 8.23 (d, *J* = 7.6 Hz, 1H), 5.81 (br s, 1H), 3.64 (tdt, *J* = 11.6, 7.9, 3.7 Hz, 1H), 2.79 – 2.67 (m, 1H), 2.55 – 2.51 (m, 1H), 2.48 (s, 3H), 1.98 – 1.92 (m, 2H), 1.92 – 1.85 (m, 3H), 1.85 – 1.77 (m, 1H), 1.55 – 1.43 (m, 2H), 1.42 – 1.31 (m, 2H). UPLC t_R = 1.78 min, Purity UV_{254} 97%. HRMS (ESI) m/z: $[M + H]^+$ Calcd for $C_{17}H_{21}F_2N_4O_2$ 351.1627; Found 351.1639.

*(1R)-2,2-Difluoro-N-[trans-4-(3-methyl-6-oxo-2,7-dihydropyrazolo[3,4-b]pyridin-4-yl)cyclohexyl]cyclopropanecarboxamide (**28**)*. Prepared using the general procedure. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.74 (br s, 1H), 11.32 (br s, 1H), 8.23 (d, *J* = 7.7 Hz, 1H), 5.82 (br s, 1H), 3.64 (tdt, *J* = 11.6, 7.9, 4.1 Hz, 1H), 2.82 – 2.68 (br m, 1H), 2.55 – 2.51 (m, 1H), 2.48 (s, 3H), 2.00 – 1.92 (m, 2H), 1.92 – 1.85 (m, 3H), 1.85 – 1.77 (m, 1H), 1.54 – 1.43 (m, 2H), 1.42 – 1.31 (m, 2H). UPLC t_R = 1.78 min, Purity UV_{254} >99%. HRMS (ESI) m/z: $[M + H]^+$ Calcd for $C_{17}H_{21}F_2N_4O_2$ 351.1627; Found 351.1632.

*N-Cyclopropyl-trans-4-(3-methyl-6-oxo-2,7-dihydropyrazolo[3,4-b]pyridin-4-yl)cyclohexanecarboxamide (**25**)*. To a solution of 4-[1,7-*bis*[(4-methoxyphenyl)methyl]-3-methyl-6-oxo-pyrazolo[3,4-*b*]pyridin-4-yl]cyclohexanecarboxylic acid **77** (40 mg, 0.078 mmol) in DMF (1 mL) was added HATU (38 mg, 0.10 mmol), cyclopropylamine (6.6 mg, 0.12 mmol) and DIPEA (40.5 µL, 0.233 mmol). The reaction was stirred at room temperature for 5 min. It was poured into 1 N HCl (10 mL) and the product was extracted with EtOAc (3 × 10 ml). The combined organic layers were washed with sat. $NaHCO_3$ (10 mL), brine (10 mL), dried over $MgSO_4$, filtered and evaporated to afford 4-[1,7-*bis*[(4-methoxyphenyl)methyl]-3-methyl-6-oxo-pyrazolo[3,4-*b*]pyridin-4-yl]-*N*-cyclopropyl-cyclohexanecarboxamide (41 mg, 90%) as a solid. A portion of this material (27 mg, 0.049 mmol) was dissolved in TFA (1 mL) and the solution was heated in a microwave synthesizer at 80 °C for 1.5 h. The reaction mixture was purified by HPLC (acidic method) to afford *N*-cyclopropyl-4-(3-methyl-6-oxo-2,7-dihydropyrazolo[3,4-*b*]pyridin-4-yl)cyclohexanecarboxamide **25** (2.3 mg, 9.4%) as the pure *trans* isomer. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.74 (br s, 1H), 11.32 (br s, 1H), 7.80 (br d, *J* = 4.3 Hz, 1H), 5.78 (br s, 1H), 2.81 – 2.68 (br m, 1H), 2.64 – 2.58 (m, 1H), 2.47 (s, 3H), 2.11 (tt, *J* = 12.0, 3.7 Hz, 1H), 1.93 – 1.85 (m, 2H), 1.84 – 1.77 (m, 2H), 1.60 – 1.49 (m, 2H), 1.42 – 1.31 (m, 2H), 0.61 – 0.56 (m, 2H), 0.39 – 0.35 (m, 2H). UPLC t_R = 1.68 min, Purity UV_{254} >99%. HRMS (ESI) m/z: $[M + H]^+$ Calcd for $C_{17}H_{23}N_4O_2$ 315.1816; Found 315.1823.

*[trans-4-(3-Methyl-6-oxo-2,7-dihydropyrazolo[3,4-b]pyridin-4-yl)cyclohexyl] (1S)-2,2-difluorocyclopropanecarboxylate (**29**)*. A mixture of 4-(4-*trans*-hydroxycyclohexyl)-3-methyl-2,7-dihydropyrazolo[3,4-*b*]pyridin-6-one **33** (100 mg, 0.404 mmol), (1*S*)-2,2-difluorocyclopropanecarboxylic acid, HATU (154 mg, 0.404 mmol) and DMAP (74 mg, 0.607 mmol) in DMF (2 mL) was stirred at rt for 15 h. The mixture was purified without workup by HPLC to afford [*trans*-4-(3-methyl-6-oxo-2,7-dihydropyrazolo[3,4-*b*]pyridin-4-yl)cyclohexyl] (1*S*)-2,2-difluorocyclopropanecarboxylate **29** (18 mg, 13%) as a white solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.75 (br s, 1H), 11.33 (br s, 1H), 5.80 (br s, 1H), 4.83 – 4.75 (m, 1H), 3.39 – 3.36 (m, 1H), 2.83 – 2.74 (m, 1H), 2.48 (s, 3H), 2.09 – 1.95 (m, 4H), 1.93 – 1.87 (m, 2H), 1.63 – 1.48 (m, 4H). UPLC t_R = 1.98 min, Purity UV_{254} 95%. HRMS (ESI) m/z: $[M + H]^+$ Calcd for $C_{17}H_{20}F_2N_3O_3$ 352.1467; Found 352.1482.

General procedure for sulfonamide formation in library format. A stock solution of 4-(4-*trans*-aminocyclohexyl)-3-methyl-2,7-dihydropyrazolo[3,4-*b*]pyridin-6-one **32** (80 mg, 0.32 mmol) in DMSO (4.0 mL) was prepared, and DIPEA (288 µL, 1.65 mmol) was added. To each sulfonyl chloride (0.12 mmol) in the library was added an aliquot of the stock solution (0.50 mL) and the resulting mixture was shaken at rt for 15 min. The reaction mixtures were purified by HPLC (acidic method) without prior work-up to afford the corresponding sulfonamides.

*N-[trans-4-(3-Methyl-6-oxo-2,7-dihydropyrazolo[3,4-b]pyridin-4-yl)cyclohexyl]cyclopropanesulfonamide (**30**)*. Prepared using the general procedure. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.30 (br s, 1H), 11.78 (br s, 1H), 7.09 (br s, 1H), 5.82 (br s, 1H), 3.22 (tt, *J* = 11.0, 4.1 Hz, 1H), 2.75 – 2.67 (m, 1H), 2.60 – 2.54 (m, 1H), 2.47 (s, 3H), 2.09 – 2.02 (m, 2H), 1.91 – 1.84 (m, 2H), 1.54 – 1.37 (m, 4H), 0.98 – 0.90 (m, 4H). UPLC t_R = 1.73 min, Purity UV_{254} >99%. HRMS (ESI) m/z: $[M + H]^+$ Calcd for $C_{16}H_{23}N_4O_3S$ 351.1485; Found 351.1513.

*1-Cyclobutyl-N-[trans-4-(3-methyl-6-oxo-2,7-dihydropyrazolo[3,4-b]pyridin-4-yl)cyclohexyl]methanesulfonamide (**31**)*. Prepared using the general procedure. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.75 (s, 1H), 11.31 (s, 1H), 7.01 (d, *J* = 7.4 Hz, 1H), 5.78 (s, 1H), 3.19 – 3.13 (m, 1H), 3.12 (d, *J* = 7.2 Hz, 2H), 2.73 – 2.63 (m, 2H), 2.46 (s, 3H), 2.15 – 2.06 (m, 2H), 2.03 – 1.96 (m, 2H), 1.91 – 1.74 (m, 6H), 1.52 – 1.34 (m, 4H). UPLC t_R = 1.89 min, Purity UV_{254} >99%. HRMS (ESI) m/z: $[M + H]^+$ Calcd for $C_{18}H_{27}N_4O_3S$ 379.1798; Found 379.1825.

*4-(4-trans-Aminocyclohexyl)-3-methyl-2,7-dihydropyrazolo[3,4-b]pyridin-6-one (**32**)*. To a solution of *tert*-butyl *N*-(4-formylcyclohexyl)carbamate **59l** (1.17 g,

5.15 mmol) in ethanol (13 mL) was added Meldrum's acid **60** (371 mg, 2.57 mmol). The reaction mixture was stirred at rt for 5 min followed by the addition of 3-methyl-1*H*-pyrazol-5-amine **43c** (250 mg, 2.57 mmol) and the resulting mixture was stirred at 60 °C overnight. After cooling to rt, the reaction mixture was reduced to half its initial volume by evaporation and ether (10 mL) was added. The resulting white precipitate was collected by filtration and washed with ether to afford *tert*-butyl *N*-[4-(3-methyl-6-oxo-2,4,5,7-tetrahydropyrazolo[3,4-*b*]pyridin-4-yl)cyclohexyl]carbamate **61l** (502 mg, 56%) as a white solid that was used without purification. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.71 (s, 1H), 10.05 (s, 1H), 6.63 (d, *J* = 8.0 Hz, 1H), 3.11 – 3.01 (m, 1H), 2.61 (t, *J* = 6.5 Hz, 1H), 2.53 – 2.49 (m, 1H), 2.35 (d, *J* = 16.8 Hz, 1H), 2.11 (s, 3H), 1.80 – 1.69 (m, 2H), 1.68 – 1.62 (m, 1H), 1.55 – 1.47 (m, 1H), 1.35 (s, 9H), 1.22 – 1.14 (m, 1H), 1.11 – 1.00 (m, 2H), 1.00 – 0.90 (m, 2H).

To a suspension of *tert*-butyl *N*-[4-(3-methyl-6-oxo-2,4,5,7-tetrahydropyrazolo[3,4-*b*]pyridin-4-yl)cyclohexyl]carbamate **61l** (502 mg, 1.44 mmol) in 1,4-dioxane (8 mL) was added DDQ (327 mg, 1.44 mmol) and the mixture was heated at 100 °C for 30 min. After cooling to rt the precipitated solid was collected by filtration and washed with ether to afford *tert*-butyl *N*-[4-(3-methyl-6-oxo-2,7-dihydropyrazolo[3,4-b]pyridin-4-yl)cyclohexyl]carbamate **32a** (498 mg, 99%) as a white solid that was used without purification. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.74 (s, 1H), 11.31 (s, 1H), 6.77 (d, *J* = 7.9 Hz, 1H), 5.81 (s, 1H), 2.75 – 2.59 (m, 1H), 2.52 – 2.49 (m, 1H), 2.46 (s, 3H), 1.96 – 1.81 (m, 4H), 1.51 – 1.40 (m, 2H), 1.39 (s, 9H), 1.36 – 1.27 (m, 2H).

The intermediate *tert*-butyl *N*-[4-(3-methyl-6-oxo-2,7-dihydropyrazolo[3,4-b]pyridin-4-yl)cyclohexyl]carbamate **32a** (498 mg, 1.44 mmol) was dissolved in 4 M HCl in 1,4-dioxane and the mixture was stirred at rt for 15 min. Evaporation to dryness afforded 4-(4-*trans*-aminocyclohexyl)-3-methyl-2,7-dihydropyrazolo[3,4-*b*]pyridin-6-one **32** as the hydrochloride salt (350 mg, 86%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.11 (br s, 3H), 5.86 (s, 1H), 3.13 – 3.01 (m, 1H), 2.80 – 2.71 (m, 1H), 2.48 (s, 3H), 2.14 – 2.03 (m, 2H), 1.97 – 1.86 (m, 2H), 1.57 – 1.44 (m, 4H). Not all exchangeable protons are visible. UPLC t_R = 0.87 min, Purity UV_{254} 95%. HRMS (ESI) m/z: $[M + H]^+$ Calcd for $C_{13}H_{19}N_4O$ 247.1553; Found 247.1576.

*4-(4-trans-Hydroxycyclohexyl)-3-methyl-2,7-dihydropyrazolo[3,4-b]pyridin-6-one (**33**)*. To solution of *trans*-4-[*tert*-butyl(dimethyl)silyl]oxycyclohexanecarbaldehyde **59m**[34] (20 g, 83 mmol) in ethanol (1.4 L) was added Meldrum's acid **60** (5.6 g, 58 mmol). The resulting mixture was stirred at rt for 15 min followed by the addition of 3-methyl-1*H*-pyrazol-5-amine **43c** (8.3 g, 58 mmol) and the resulting mixture was stirred at 90 °C for 3 h. After cooling to rt, the mixture was concentrated under reduced pressure and the solid thus obtained was collected by filtration, washed with water and diethyl ether, and dried under vacuum to afford 4-[4-[*tert*-butyl(dimethyl)-silyl]oxycyclohexyl]-3-methyl-2,4,5,7-tetrahydropyrazolo[3,4-*b*]pyridin-6-one **61m** (10 g, 45%) as an off-white solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.71 (s, 1H), 10.05 (s, 1H), 3.50 – 3.42 (m, 1H), 3.40 – 3.34 (m, 1H), 2.68 – 2.57 (m, 1H), 2.56 – 2.51 (m, 1H), 2.11 (s, 3H), 1.82 – 1.73 (m, 2H), 1.67 – 1.47 (m, 2H), 1.28 – 1.21 (m, 1H), 1.16 – 1.07 (m, 2H), 1.02 – 0.92 (m, 2H), 0.86 (s, 9H), 0.02 (s, 6H).

To a solution of 4-[4-[*tert*-butyl(dimethyl)silyl]oxycyclohexyl]-3-methyl-2,4,5,7-tetrahydropyrazolo[3,4-*b*]pyridin-6-one **61m** (170 mg, 0.468 mmol) in 1,4-dioxane (5 mL) was added DDQ (106 mg, 0.468 mmol) and the mixture was stirred at 100 °C for 30 min. After cooling to rt, the precipitate was collected by filtration, washed with 1,4-dioxane and dried under vacuum to afford 4-[4-[*tert*-butyl(dimethyl)silyl]oxycyclohexyl]-3-methyl-2,7-dihydropyrazolo[3,4-*b*]pyridin-6-one **33a** (100 mg, 59%) as an off-white solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.85 (s, 1H), 11.40 (s, 1H), 5.78 (br s, 1H), 3.75 – 3.65 (m, 1H), 2.47 (s, 3H), 1.93 – 1.91 (m, 2H), 1.87 – 1.81 (m, 2H), 1.50 – 1.39 (m, 4H), 0.87 (s, 9H), 0.06 (s, 6H).

To a solution of 4-[4-[*tert*-butyl(dimethyl)silyl]oxycyclohexyl]-3-methyl-2,7-dihydropyrazolo[3,4-*b*]pyridin-6-one **33a** (100 mg, 0.275 mmol) in DCM (5 mL) at 0 °C was added 1 M TBAF in THF (0.55 mL, 0.55 mmol) at 0 °C. The resulting reaction mass was stirred at 40 °C for 16 h. Volatiles were evaporated and the residue was triturated with saturated $NaHCO_3$ solution to give a solid. The solid was filtered, washed with ether and dried under vacuum to afford 4-(4-*trans*-hydroxycyclohexyl)-3-methyl-2,7-dihydropyrazolo[3,4-*b*]pyridin-6-one **33** (56 mg, 40%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.73 (s, 1H), 11.31 (s, 1H), 5.78 (s, 1H), 4.61 (d, *J* = 4.4 Hz, 1H), 3.51 – 3.41 (m, 1H), 2.77 – 2.62 (m, 1H), 2.47 (s, 3H), 1.98 – 1.89 (m, 2H), 1.87 – 1.77 (m, 2H), 1.48 – 1.21 (m, 4H). UPLC t_R = 1.54 min, Purity UV_{254} 98%. HRMS (ESI) m/z: $[M + H]^+$ Calcd for $C_{13}H_{18}N_3O_2$ 248.1394; Found 248.1382.

General procedure for amide formation in library format. One acid, multiple amines. A stock solution of 2-[*trans*-4-(3-methyl-6-oxo-2,7-dihydropyrazolo[3,4-*b*]pyridin-4-yl)cyclohexyl]acetic acid **78** (95.0 mg, 0.328 mmol) in DMF (4.75 mL) was prepared, and an aliquot (0.25 mL, 0.017 mmol) was added to each amine (0.0346 mmol) in the library. A stock solution of HATU (124 mg, 0.326 mmol) in DMF (4.75 mL) was prepared, and an aliquot (0.25 mL, 0.017 mmol) was added to each of the reaction mixtures. Finally, DIPEA (9.0 µL, 0.052 mmol) was added to each of the reaction mixtures, which were then shaken at rt for 15 min. The reaction mixtures were purified by HPLC without prior work-up to afford the corresponding amides.

*1-[2-[trans-4-(3-Methyl-6-oxo-2,7-dihydropyrazolo[3,4-b]pyridin-4-yl)cyclohexyl]acetyl]azetidine-3-carbonitrile (**34**)*. Prepared using the general procedure. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.72 (br s, 1H), 11.29 (br s, 1H), 5.78 (br s, 1H), 4.40 (t, *J* = 8.8 Hz, 1H), 4.32 (dd, *J* = 8.4, 6.0 Hz, 1H), 4.13 (t, *J* = 9.3 Hz, 1H), 3.98 (dd, *J* = 9.4, 6.1 Hz, 1H), 3.76 (tt, *J* = 9.1, 6.0 Hz, 1H), 2.75 – 2.64 (m, 1H), 2.45 (s, 3H), 2.03 – 1.93 (m, 2H), 1.90 – 1.79 (m, 4H), 1.78 – 1.68 (m, 1H), 1.46 – 1.32 (m, 2H), 1.21 – 1.08 (m, 2H). UPLC t_R = 1.72 min, Purity UV_{254} 81%. HRMS (ESI) m/z: $[M + H]^+$ Calcd for $C_{19}H_{24}N_5O_2$ 354.1925; Found 354.1943.

*1-[2-[trans-4-(3-Methyl-6-oxo-2,7-dihydropyrazolo[3,4-b]pyridin-4-yl)cyclohexyl]acetyl]pyrrolidine-3-carbonitrile (**35**)*. Prepared using the general procedure. Two rotamers

around the amide bond are visible in the NMR spectrum. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.73 (br s, 1H), 11.29 (br s, 1H), 5.78 (br s, 1H), 3.78 (dd, *J* = 10.3, 7.2 Hz, 0.5H), 3.67 (dd, *J* = 10.3, 6.0 Hz, 0.5H), 3.62 (dd, *J* = 11.8, 7.4 Hz, 0.5H), 3.59 – 3.35 (m, 3.5H), 2.75 – 2.64 (br m, 1H), 2.46 (s, 3H), 2.33 – 2.26 (m, 0.5H), 2.24 – 2.14 (m, 3H), 2.11 – 2.03 (m, 0.5H), 1.90 – 1.74 (m, 5H), 1.47 – 1.33 (m, 2H), 1.23 – 1.10 (m, 2H). UPLC t_R = 1.76 min, Purity UV_{254} >99%. HRMS (ESI) m/z: $[M + H]^+$ Calcd for $C_{20}H_{26}N_5O_2$ 368.2081; Found 368.2093.

*4-[trans-4-[2-(3-Benzylazetidin-1-yl)-2-oxo-ethyl]cyclohexyl]-3-methyl-2,7-dihydropyrazolo[3,4-b]-pyridin-6-one (**36**)*. Prepared using the general procedure. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.72 (br s, 1H), 11.32 (br s, 1H), 7.33 – 7.26 (m, 2H), 7.24 – 7.17 (m, 3H), 5.80 (br s, 1H), 4.15 (t, *J* = 7.9 Hz, 1H), 3.88 (t, *J* = 8.6 Hz, 1H), 3.81 (dd, *J* = 8.3, 4.6 Hz, 1H), 3.55 (dd, *J* = 9.5, 4.8 Hz, 1H), 2.89 – 2.81 (m, 3H), 2.75 – 2.67 (m, 1H), 2.46 (s, 3H), 1.95 (d, *J* = 6.9 Hz, 2H), 1.88 – 1.77 (m, 4H), 1.76 – 1.67 (m, 1H), 1.44 – 1.34 (m, 2H), 1.18 – 1.09 (m, 2H). UPLC t_R = 2.04 min, Purity UV_{254} 97%. HRMS (ESI) m/z: $[M + H]^+$ Calcd for $C_{25}H_{31}N_4O_2$ 419.2442; Found 419.2459.

*3-Methyl-4-[trans-4-[2-oxo-2-(3-phenylpyrrolidin-1-yl)ethyl]cyclohexyl]-2,7-dihydropyrazolo[3,4-b]pyridin-6-one (**37**)*. Prepared using the general procedure. Two rotamers around the amide bond are visible in the NMR spectrum. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.73 (br s, 1H), 11.30 (br s, 1H), 7.38 – 7.28 (m, 4H), 7.26 – 7.20 (m, 1H), 5.79 (br s, 1H), 3.93 (dd, *J* = 9.4, 7.1 Hz, 0.5H), 3.84 (dd, *J* = 11.5, 7.6 Hz, 0.5H), 3.70 – 3.64 (m, 0.5H), 3.64 – 3.58 (m, 0.5H), 3.52 (td, *J* = 9.8, 6.8 Hz, 0.5H), 3.46 – 3.40 (m, 0.5H), 3.40 – 3.34 (m, 0.5H), 3.20 (dd, *J* = 11.4, 9.3 Hz, 0.5H), 2.78 – 2.64 (m, 1H), 2.47 (s, 1.5H), 2.45 (s, 1.5H), 2.33 – 2.26 (m, 0.5H), 2.24 – 2.17 (m, 3H), 2.06 – 1.97 (m, 0.5H), 1.96 – 1.77 (m, 6H), 1.47 – 1.33 (m, 2H), 1.26 – 1.10 (m, 2H). UPLC t_R = 2.00 min, Purity UV_{254} 94%. HRMS (ESI) m/z: $[M + H]^+$ Calcd for $C_{25}H_{31}N_4O_2$ 419.2442; Found 419.2460.

*4-[trans-4-[2-(4,4-Dimethyl-1-piperidyl)-2-oxo-ethyl]cyclohexyl]-3-methyl-2,7-dihydropyrazolo[3,4-b]pyridin-6-one (**38**)*. Prepared using the general procedure. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.73 (br s, 1H), 11.29 (br s, 1H), 5.78 (br s, 1H), 3.47 – 3.38 (m, 4H), 2.76 – 2.63 (m, 1H), 2.46 (s, 3H), 2.23 (d, *J* = 6.9 Hz, 2H), 1.89 – 1.80 (m, 4H), 1.79 – 1.70 (m, 1H), 1.45 – 1.33 (m, 2H), 1.32 – 1.27 (m, 2H), 1.24 – 1.19 (m, 2H), 1.19 – 1.10 (m, 2H), 0.94 (s, 6H). UPLC t_R = 2.04 min, Purity UV_{254} >99%. HRMS (ESI) m/z: $[M + H]^+$ Calcd for $C_{22}H_{33}N_4O_2$ 385.2598; Found 385.2609.

*Norbornane acetonitriles **39** – **42.*** To a solution of 2-[(1*S**,4*S**,5*R**)-5-formylnorbornan-2-ylidene]acetonitrile **59o** (0.750 g, 4.61 mmol) in ethanol (30 mL) was added Meldrum's acid **60** (0.670 g, 4.6 mmol), the mixture was stirred at rt for 15 min, and 3-methyl-1*H*-pyrazol-5-amine **43c** (0.450 g, 4.6 mmol) was added. The resulting mixture was stirred at 80 °C for 3 h. After cooling to rt, the mixture was concentrated under reduced pressure and the residue was washed with ether. The solid thus obtained was dried under vacuum to afford 2-[(1*S**,4*S**,5*R**)-5-(6-oxo-2,4,5,7-tetrahydropyrazolo[3,4-*b*]pyridin-4-yl)norbornan-2-ylidene]acetonitrile **61o** (0.7 g, 53%) as an off-white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.77 – 11.52 (m, 1H), 10.25 – 9.98 (m, 1H), 5.34 – 5.20 (m, 1H), 3.17 (s, 1H), 2.66 – 2.53 (m, 2H), 2.26 (br s, 3H), 2.17 (d, *J* = 4.4 Hz, 3H), 2.00 (br d, *J* = 17.6 Hz, 1H), 1.56 – 1.18 (m, 3H), 1.20 – 1.08 (m, 2H).

To a solution of 2-[(1*S**,4*S**,5*R**)-5-(6-oxo-2,4,5,7-tetrahydropyrazolo[3,4-*b*]pyridin-4-yl)norbornan-2-ylidene]acetonitrile **61o** (0.700 g, 2.48 mmol) in 1,4-dioxane (20 mL) was added DDQ (0.845 g, 3.73 mmol). The resulting mixture was stirred at 100 °C for 3 h. After cooling to rt, the mixture was concentrated under reduced pressure and the residue was purified by column chromatography (silica gel column, DCM:MeOH 95:5) to afford 2-[(1*S**,4*S**,5*R**)-5-(6-oxo-2,7-dihydropyrazolo[3,4-*b*]pyridin-4-yl)norbornan-2-ylidene]acetonitrile **39a** (0.500 g, 71%) as an off-white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.03 – 12.61 (m, 1H), 11.39 (br d, *J* = 9.2 Hz, 1H), 5.97 – 5.77 (m, 1H), 5.49 – 5.23 (m, 1H), 3.23 (br d, *J* = 3.7 Hz, 1H), 3.07 (br d, *J* = 4.0 Hz, 1H), 2.54 – 2.39 (m, 3H), 2.18 – 1.77 (m, 2H), 1.72 – 1.41 (m, 4H), 1.31 – 1.10 (m, 1H).

A suspension of 2-[(1*S**,4*S**,5*R**)-5-(6-oxo-2,7-dihydropyrazolo[3,4-*b*]pyridin-4-yl)norbornan-2-ylidene]acetonitrile **39a** (0.500 g, 2.14 mmol) and 5% Pt/C (0.500 g, 50% of moisture) in methanol (30 mL) was stirred under H_2 (1 atm.) at rt for 16 h. The reaction mixture was filtered through a celite pad and the pad was washed with methanol. The filtrate was concentrated under reduced pressure and the residue was subjected to chiral SFC purification (Column: Chiralpak AD-H 30 × 250 mm, 5 μm; CO_2:MeOH 35:65 wt/wt; total flow: 90.0 g/min; back pressure: 100 bar; detection: UV 296 nm; stack time: 12.5 min; load per injection: 12.5 mg dissolved in 1.25 mL MeOH; number of injections: 45). Compounds **41** and **42** were isolated as the pure enantiomers, but compounds **39** and **40** were obtained as a mixture. The enantiomeric purities of **41** and **42** were determined by analytical chiral SFC (Column: Chiralpak AD-H 4.6 × 250 mm, 5 μm; CO_2:MeOH 60:40 wt/wt, column temperature: 25 °C, total flow: 4 g/min). The mixture of compounds **39** and **40** was subjected to a second round of chiral SFC purification (Column: Chiralpak IC 30 × 250 mm, 5 μm; CO_2:co-solvent 60:40 wt/wt, where the co-solvent is 0.5% DEA in MeOH; total flow: 90.0 g/min; back pressure: 100 bar; detection: UV 214 nm; stack time: 16.5 min; load per injection: 5.04 mg dissolved in MeCN + MeOH; number of injections: 20), which afforded compounds **39** and **40** as the pure enantiomers. The enantiomeric purities of **39** and **40** were determined by analytical chiral SFC (Column: Chiralpak IC 4.6 × 250 mm, 5 μm; CO_2:co-solvent 60:40 wt/wt, where the co-solvent is 0.5% DEA in MeOH, column temperature: 30 °C, total flow: 4 g/min). The relative stereochemistry of compounds **39** to **42** was assigned from ^{1}H NMR NOE experiments, see supporting information for details. Absolute configurations were not determined.

*[(1S,2S,4S,5R)-5-(3-Methyl-6-oxo-1,2,7-triaza-2,7-dihydroinden-4-yl)-2-norbornanyl]acetonitrile (**39**).* Yield 30 mg, 6.0%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.05 (br s, 2H), 5.78 (br s, 1H), 2.93 (br t, *J* = 7.3 Hz, 1H), 2.53 – 2.49 (m, 1H), 2.48 (s, 3H), 2.43 (dd, *J* = 16.9, 7.7 Hz, 1H), 2.31 (br d, *J* = 4.0 Hz, 1H), 2.16 (br s, 1H), 1.95 – 1.89 (m, 1H), 1.79 – 1.73 (m, 1H), 1.72 – 1.62 (m, 2H), 1.44 – 1.40 (m, 1H), 1.35

– 1.30 (m, 1H), 1.22 (dt, *J* = 12.5, 4.5 Hz, 1H). Chiral SFC t_R = 14.28 min, 94% ee. UPLC t_R = 1.82 min, Purity UV_{254} 95%. HRMS (ESI) m/z: $[M + H]^+$ Calcd for $C_{16}H_{19}N_4O$ 283.1553; Found 283.1566.

*[(1R,2R,4R,5S)-5-(3-Methyl-6-oxo-1,2,7-triaza-2,7-dihydroinden-4-yl)-2-norbornanyl]acetonitrile (**40**).* Yield 11 mg, 2.2%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.72 (br s, 1H), 11.30 (br s, 1H), 5.78 (br s, 1H), 2.92 (br s, 1H), 2.53 – 2.49 (m, 1H), 2.48 (s, 3H), 2.43 (dd, *J* = 17.0, 7.7 Hz, 1H), 2.31 (br d, *J* = 3.6 Hz, 1H), 2.16 (br d, *J* = 2.7 Hz, 1H), 1.95 – 1.89 (m, 1H), 1.79 – 1.73 (m, 1H), 1.72 – 1.62 (m, 2H), 1.44 – 1.40 (m, 1H), 1.35 – 1.30 (m, 1H), 1.22 (dt, *J* = 12.5, 4.5 Hz, 1H). Chiral SFC t_R = 10.28 min, >99% ee. UPLC t_R = 1.81 min, Purity UV_{254} 96%. HRMS (ESI) m/z: $[M + H]^+$ Calcd for $C_{16}H_{19}N_4O$ 283.1553; Found 283.1556.

*[(1S,2R,4S,5R)-5-(3-Methyl-6-oxo-1,2,7-triaza-2,7-dihydroinden-4-yl)-2-norbornanyl]acetonitrile (**41**).* Yield 80 mg, 16%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.70 (br s, 1H), 11.29 (br s, 1H), 5.77 (br s, 1H), 2.94 (br s, 1H), 2.67 (dd, *J* = 16.8, 8.1 Hz, 1H), 2.56 (dd, *J* = 16.9, 8.0 Hz, 1H), 2.50 (s, 3H), 2.32 (br s, 1H), 2.28 – 2.15 (m, 2H), 2.01 – 1.91 (m, 1H), 1.90 – 1.82 (m, 1H), 1.62 – 1.53 (m, 1H), 1.52 – 1.45 (m, 1H), 1.38 – 1.31 (m, 1H), 1.05 – 0.96 (m, 1H). Chiral SFC t_R = 4.38 min, >99% ee. UPLC t_R = 1.81 min, Purity UV_{254} 94%. HRMS (ESI) m/z: $[M + H]^+$ Calcd for $C_{16}H_{19}N_4O$ 283.1553; Found 283.1567.

*[(1R,2S,4R,5S)-5-(3-Methyl-6-oxo-1,2,7-triaza-2,7-dihydroinden-4-yl)-2-norbornanyl]acetonitrile (**42**).* Yield 80 mg, 16%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.96 (br s, 2H), 5.79 (br s, 1H), 2.96 (br t, *J* = 7.2 Hz, 1H), 2.67 (dd, *J* = 16.9, 8.0 Hz, 1H), 2.56 (dd, *J* = 16.8, 8.0 Hz, 1H), 2.50 (s, 3H), 2.32 (br s, 1H), 2.28 – 2.15 (m, 2H), 2.01 – 1.91 (m, 1H), 1.90 – 1.82 (m, 1H), 1.62 – 1.53 (m, 1H), 1.52 – 1.45 (m, 1H), 1.38 – 1.31 (m, 1H), 1.05 – 0.96 (m, 1H). Chiral SFC t_R = 3.65 min, >99% ee. UPLC t_R = 1.82 min, Purity UV_{254} >99%. HRMS (ESI) m/z: $[M + H]^+$ Calcd for $C_{16}H_{19}N_4O$ 283.1553; Found 283.1555.

*Methyl 4-(3-methyl-6-oxo-2,4,5,7-tetrahydropyrazolo[3,4-b]pyridin-4-yl)cyclohexanecarboxylate (**61n**).* Meldrum's acid **60** (423 mg, 2.94 mmol) and methyl *trans*-4-formylcyclohexanecarboxylate **59n** (500 mg, 2.94 mmol) were mixed in pyridine (5 mL). Piperidine (29 µL, 0.29 mmol) was added and the reaction mixture was stirred at room temperature for 3 h. To the reaction mixture was added ethanol (10 mL) and 3-methyl-1*H*-pyrazol-5-amine **43c** (285 mg, 2.94 mmol). The reaction mixture was heated at 70 °C for 1 h. After cooling to rt, volatiles were evaporated and the residue was purified by chromatography (ISCO CombiFlash, 40 g silica gel column, EtOAc:MeOH 100:0 → 90:10) to give methyl 4-(3-methyl-6-oxo-2,4,5,7-tetrahydropyrazolo[3,4-*b*]pyridin-4-yl)cyclohexanecarboxylate **61n** (200 mg, 23%) as a yellow solid (mixture of isomers, *cis*:*trans* ca. 20:80). ^{1}H NMR for the trans isomer (600 MHz, DMSO-d_6) δ 11.71 (s, 1H), 10.05 (s, 1H), 3.56 (s, 3H), 2.68 – 2.59 (m, 1H), 2.54 – 2.51 (m, 1H), 2.38 – 2.31 (m, 1H), 2.21 – 2.14 (m, 1H), 2.11 (s, 3H), 1.93 – 1.83 (m, 2H), 1.74 – 1.65 (m, 1H), 1.60 – 1.54 (m, 1H), 1.32 – 1.18 (m, 3H), 1.03 – 0.92 (m, 2H).

*6-Chloro-4-cyclohexyl-3-methyl-1H-pyrazolo[3,4-b]pyridine-5-carbaldehyde (**62**).* To a suspension of 4-cyclohexyl-3-methyl-2,4,5,7-tetrahydropyrazolo[3,4-*b*]pyridin-6-one **61a** (854 mg, 3.66 mmol) in DMF (7 mL) was slowly added $POCl_3$ (0.716 mL, 7.69 mmol) at rt (exothermic) in a closed microwave vial. The mixture became yellow and homogenous. It was stirred at 100 °C for 3 h to produce a dark red solution. After cooling to rt, volatiles were evaporated and the residual thick syrup was stirred in water (20 mL) overnight. The resulting yellow precipitate was filtered off and dried. The so obtained crude intermediate was suspended in MeCN (50 mL) and MnO_2 (3 g) was added. The mixture was stirred at rt for 2 hours, filtered and evaporated. The residue was purified by chromatography (ISCO CombiFlash, silica gel column, heptane:EtOAc) to afford 6-chloro-4-cyclohexyl-3-methyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbaldehyde **62** (150 mg, 15%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.93 (br s, 1H), 10.65 (s, 1H), 3.62 (br t, *J* = 12.5 Hz, 1H), 2.79 (s, 3H), 2.09 – 1.87 (m, 4H), 1.87 – 1.72 (m, 3H), 1.52 – 1.31 (m, 3H).

*2-[4-[1,7-bis[(4-Methoxyphenyl)methyl]-3-methyl-6-oxo-pyrazolo[3,4-b]pyridin-4-yl]cyclohexyl]acetonitrile (**70**).* To a solution of 2-[4-(3-methyl-6-oxo-2,4,5,7-tetrahydro-pyrazolo[3,4-*b*]pyridin-4-yl)cyclohexyl]acetonitrile **61j** (4.00 g, 14.7 mmol) in DMF (20 mL) was added Cs_2CO_3 (14.4 g, 44.1 mmol) followed by 4-methoxybenzyl chloride (4.3 mL, 32 mmol). The resulting reaction mixture was stirred at rt for 16 h. The mixture was then diluted with water and extracted with EtOAc (2 × 100 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to afford 2-[4-[1,7-*bis*[(4-methoxyphenyl)methyl]-3-methyl-6-oxo-4,5-dihydropyrazolo[3,4-*b*]pyridin-4-yl]cyclohexyl]acetonitrile **69** (4.0 g, 53%), which was used without purification.

To a solution of 2-[4-[1,7-*bis*[(4-methoxyphenyl)-methyl]-3-methyl-6-oxo-4,5-dihydropyrazolo[3,4-*b*]pyridin-4-yl]cyclohexyl]acetonitrile **69** (4.0 g, 7.8 mmol) in 1,4-dioxane (30 mL) was added DDQ (1.8 g, 7.8 mmol). The resulting mixture was stirred at 100 °C for 16 h. The mixture was concentrated under reduced pressure and the residue was dissolved in 1,4-dioxane and filtered. The filtrate was concentrated under reduced pressure. The crude product was purified by column chromatography (silica gel column, hexane:EtOAc 100:0 → 50:50) to afford 2-[4-[1,7-*bis*[(4-methoxyphenyl)methyl]-3-methyl-6-oxo-pyrazolo[3,4-*b*]pyridin-4-yl]cyclohexyl]acetonitrile **70** (2.0 g, 50%) as a brown solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.29 (d, *J* = 8.7 Hz, 2H), 7.16 (d, *J* = 8.9 Hz, 2H), 6.94 – 6.86 (m, 2 H) 6.83 – 6.78 (m, 2H), 5.96 (s, 1H), 5.32 (s, 2H), 5.09 (s, 2H), 3.72 (s, 3H), 3.71 – 3.68 (m, 3H), 2.77 (br t, *J* = 11.8 Hz, 1H), 2.52 (s, 3H), 2.50 (s, 2H), 1.86 (br d, *J* = 11.0 Hz, 4H), 1.68 (ddd, *J* = 8.8, 5.7, 3.1 Hz, 1H), 1.49 – 1.38 (m, 2H), 1.30 – 1.22 (m, 2H).

*4-[4-(2-Hydroxyethyl)cyclohexyl]-1,7-bis[(4-methoxyphenyl)methyl]-3-methyl-pyrazolo[3,4-b]pyridin-6-one (**72**).* A solution of 2-[4-[1,7-*bis*[(4-methoxyphenyl)-methyl]-3-methyl-6-oxo-pyrazolo[3,4-*b*]pyridin-4-yl]cyclohexyl]acetonitrile **70** (600 mg, 1.18 mmol) in toluene (10 mL) was cooled to 0 °C, 1.0 M DIBAL-H in hexane (2.35 mL, 2.35 mmol) was added *via* syringe and the mixture was stirred for 2 h at 0 °C. The reaction was

quenched with sat. potassium tartrate and extracted with EtOAc (2 × 50 mL). The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure to afford 2-[4-[1,7-*bis*[(4-methoxyphenyl)methyl]-3-methyl-6-oxo-pyrazolo[3,4-*b*]pyridin-4-yl]cyclohexyl]acetaldehyde **71** (500 mg, 82%) as a colorless oil that was used without purification.

To a solution of 2-[4-[1,7-*bis*[(4-methoxyphenyl)-methyl]-3-methyl-6-oxo-pyrazolo[3,4-*b*]pyridin-4-yl]cyclohexyl]acetaldehyde **71** (3.5 g, 6.8 mmol) in THF (70 mL) was added $NaBH_4$ (1.89 g, 50.0 mmol) slowly at 0 °C. The resulting mixture was stirred at rt for 2 h. The reaction was quenched with sat. NH_4Cl at 0 °C and extracted with EtOAc (2 × 100 mL). The combined organic layers were dried over Na_2SO_4, concentrated under reduced pressure and the residue was purified by column chromatography (silica gel column, petroleum ether:EtOAc 80:20) to afford 4-[4-(2-hydroxyethyl)cyclohexyl]-1,7-*bis*[(4-methoxyphenyl)methyl]-3-methyl-pyrazolo[3,4-*b*]pyridin-6-one **72** (2.5 g, 71%) as a light yellow oil. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.29 (d, *J* = 8.8 Hz, 2H), 7.15 (d, *J* = 8.7 Hz, 2H), 6.89 (s, 2H), 6.81 (d, *J* = 8.8 Hz, 2H), 5.94 (s, 1H), 5.31 (s, 2H), 5.09 (s, 2H), 3.72 (s, 3H), 3.70 (s, 3H) 3.48 – 3.45 (m, 1H), 2.80 – 2.71 (m, 1H), 2.55 (s, 3H), 2.43 – 2.41 (m, 2H), 1.83 – 1.82 (m, 4H), 1.40 – 1.33 (m, 4H), 1.14 – 1.05 (m, 3H).

*2-[4-[1,7-bis[(4-Methoxyphenyl)methyl]-3-methyl-6-oxo-pyrazolo[3,4-b]pyridin-4-yl]cyclohexyl]ethyl methanesulfonate (**73**).* To a solution of 4-[4-(2-hydroxyethyl)cyclohexyl]-1,7-*bis*[(4-methoxyphenyl)-methyl]-3-methyl-pyrazolo[3,4-*b*]pyridin-6-one **72** (150 mg, 0.29 mmol) in DCM (10 mL) was added triethylamine (0.13 mL, 0.87 mmol) at 0 °C. After 5 min, methanesulfonyl chloride (33 µL, 0.43 mmol) was added. The resulting mixture was stirred at rt for 30 min. The reaction was quenched with sat. $NaHCO_3$ and extracted with DCM (2 × 50 mL). The combined organic layers were washed with water (30 mL), brine (50 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography (silica gel column, DCM:MeOH 100:0 → 98:2) to afford 2-[4-[1,7-*bis*[(4-methoxyphenyl)methyl]-3-methyl-6-oxo-pyrazolo[3,4-*b*]pyridin-4-yl]cyclohexyl]ethyl methanesulfonate **73** (120 mg, 69%) as a pale yellow viscous oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.52 (d, *J* = 8.8 Hz, 2H), 7.09 (d, *J* = 8.8 Hz, 2H), 6.84 (d, *J* = 8.41 Hz, 2H), 6.79 (d, *J* = 8.8 Hz, 2H), 6.18 (s, 1H), 5.27 (d, *J* = 4.8 Hz, 4H), 4.29 (t, *J* = 6.8 Hz, 2H), 3.79 (s, 3H), 3.76 (s, 3H), 3.01 (s, 3H), 2.65 (br t, *J* = 12.1 Hz, 1H), 2.42 (s, 3H), 1.92 (br d, *J* = 11.3 Hz, 4H), 1.71 (q, *J* = 6.6 Hz, 2H), 1.43 (br d, *J* = 10.6 Hz, 3H), 1.18 – 1.02 (m, 2H).

*3-[4-[1,7-bis[(4-Methoxyphenyl)methyl]-3-methyl-6-oxo-pyrazolo[3,4-b]pyridin-4-yl]cyclohexyl]propanenitrile (**74**).* To a solution of 2-[4-[1,7-*bis*[(4-methoxyphenyl)methyl]-3-methyl-6-oxo-pyrazolo[3,4-*b*]pyridin-4-yl]cyclohexyl]-ethyl methanesulfonate **73** (120 mg, 0.202 mmol) in DMF (10 mL) was added NaCN (11.8 mg, 0.241 mmol) at rt. The resulting reaction mixture was stirred at 90 °C for 16 h. The mixture was cooled to rt, diluted with water and extracted with EtOAc (2 × 30 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography (silica gel column, DCM:MeOH 100:0 → 98:2) to afford 3-[4-[1,7-*bis*[(4-methoxyphenyl)methyl]-3-methyl-6-oxo-pyrazolo[3,4-*b*]pyridin-4-yl]cyclohexyl]propanenitrile **74** (70 mg, 66%) as a pale yellow viscous oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.52 (d, *J* = 8.8 Hz, 2H), 7.09 (d, *J* = 8.8 Hz, 2H), 6.84 (d, *J* = 8.8 Hz, 2H), 6.79 (d, *J* = 8.8 Hz, 2H), 6.14 (s, 1H), 5.27 (d, *J* = 4.4 Hz, 4H), 3.79 (s, 3H), 3.76 (s, 3H), 2.65 (br t, *J* = 12.1 Hz, 1H), 2.42 (s, 3H), 2.37 (t, *J* = 7.3 Hz, 2H), 1.93 (d, *J* = 11.0 Hz, 4H), 1.68 – 1.59 (m, 2H), 1.52 – 1.41 (m, 3H), 1.15 – 1.03 (m, 2H).

*Methyl 4-[1,7-bis[(4-methoxyphenyl)methyl]-3-methyl-6-oxo-4,5-dihydropyrazolo[3,4-b]pyridin-4-yl]cyclohexanecarboxylate (**75**).* To a solution of 4-(3-methyl-6-oxo-2,4,5,7-tetrahydropyrazolo[3,4-*b*]pyridin-4-yl)cyclohexanecarboxylate **61n** (540 mg, 1.85 mmol) in DMF (25 mL) was added Cs_2CO_3 (1.51 g, 4.63 mmol) followed by 4-methoxybenzyl chloride (578 µL, 4.26 mmol). The reaction was stirred at rt for 3 h. A second portion of 4-methoxybenzyl chloride (58 mg, 0.37 mmol) was added and the reaction was stirred overnight at rt. Water (40 mL) was added and the product was extracted with EtOAc (4 × 20 mL). The combined organic layers were washed with brine (20 mL), dried over $MgSO_4$, filtered and evaporated. The residue was purified by chromatography (ISCO CombiFlash, 40 g silica gel column, heptane:EtOAc 100:0 → 0:100) to afford methyl 4-[1,7-*bis*[(4-methoxyphenyl)methyl]-3-methyl-6-oxo-4,5-dihydropyrazolo[3,4-*b*]pyridin-4-yl]cyclohexanecarboxylate **75** (903 mg, 92%) as a mixture of isomers, *cis*:*trans* ca. 20:80. ^{1}H NMR for the *trans* isomer (600 MHz, $CDCl_3$) δ 7.42 – 7.37 (m, 2H), 7.03 – 6.99 (m, 2H), 6.86 – 6.81 (m, 2H), 6.80 – 6.73 (m, 2H), 5.12 – 5.05 (m, 3H), 4.90 – 4.84 (m, 1H), 3.80 (s, 3H), 3.77 (s, 3H), 3.63 (s, 3H), 2.73 – 2.65 (m, 2H), 2.59 – 2.55 (m, 1H), 2.11 – 2.02 (m, 4H), 1.90 – 1.80 (m, 2H), 1.65 – 1.59 (m, 1H), 1.54 – 1.47 (m, 1H), 1.34 – 1.17 (m, 3H), 0.95 – 0.77 (m, 2H).

*Methyl 4-[1,7-bis[(4-methoxyphenyl)methyl]-3-methyl-6-oxo-pyrazolo[3,4-b]pyridin-4-yl]cyclohexanecarboxylate (**76**).* To a solution of methyl 4-[1,7-*bis*[(4-methoxyphenyl)methyl]-3-methyl-6-oxo-4,5-dihydropyrazolo[3,4-*b*]pyridin-4-yl]cyclohexanecarboxylate **75** (794 mg, 1.49 mmol) in 1,4-dioxane (50 mL) was added DDQ (576 mg, 2.54 mmol) and the reaction mixture was heated at 100 °C for 1 h. After cooling to rt, volatiles were removed by rotary evaporation and the residue was purified by chromatography (ISCO CombiFlash, 40 g silica gel column, heptane:EtOAc 100:0 → 0:100) to afford methyl 4-[1,7-*bis*[(4-methoxyphenyl)methyl]-3-methyl-6-oxo-pyrazolo[3,4-*b*]pyridin-4-yl]cyclohexanecarboxylate **76** (810 mg, 87%) as a semi-solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.53 – 7.48 (m, 2H), 7.11 – 7.06 (m, 2H), 6.86 – 6.82 (m, 2H), 6.80 – 6.76 (m, 2H), 6.17 (s, 1H), 5.29 (s, 2H), 5.27 (s, 2H), 3.79 (s, 3H), 3.76 (s, 3H), 3.68 (s, 3H), 2.75 – 2.67 (m, 1H), 2.44 (s, 3H), 2.38 (tt, *J* = 12.2, 3.7 Hz, 1H), 2.16 – 2.09 (m, 2H), 2.00 – 1.93 (m, 2H), 1.62 – 1.51 (m, 2H), 1.50 – 1.39 (m, 2H).

*4-[1,7-bis[(4-Methoxyphenyl)methyl]-3-methyl-6-oxo-pyrazolo[3,4-b]pyridin-4-yl]cyclohexanecarboxylic acid (**77**).* A solution of $LiOH \cdot H_2O$ (29.7 mg, 0.709 mmol) in

water (1 mL) was added to a solution of methyl 4-[1,7-*bis*[(4-methoxyphenyl)methyl]-3-methyl-6-oxo-pyrazolo[3,4-*b*]pyridin-4-yl]cyclohexanecarboxylate **76** (289 mg, 0.545 mmol) in THF (5 mL). The reaction mixture was stirred overnight at rt and then at 40 °C for 3 days. The reaction mixture was poured into water (20 mL) and the aqueous phase was washed with EtOAc (3 × 10 mL). The aqueous phase was then acidified with 4 M HCl (pH = 3) and the product was extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with brine (10 mL), dried over $MgSO_4$, filtered and evaporated to afford 4-[1,7-*bis*[(4-methoxyphenyl)methyl]-3-methyl-6-oxo-pyrazolo[3,4-*b*]pyridin-4-yl]cyclohexanecarboxylic acid **77** (142 mg, 50%), which was used without purification in the next step. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.06 (br s, 1H), 7.31 – 7.27 (m, 2H), 7.17 – 7.13 (m, 2H), 6.90 – 6.86 (m, 2H), 6.83 – 6.79 (m, 2H), 5.95 (s, 1H), 5.32 (s, 2H), 5.09 (s, 2H), 3.72 (s, 3H), 3.70 (s, 3H), 2.83 – 2.76 (m, 1H), 2.52 (s, 3H), 2.27 (tt, *J* = 12.0, 3.6 Hz, 1H), 2.02 – 1.95 (m, 2H), 1.90 – 1.83 (m, 2H), 1.55 – 1.37 (m, 4H).

*2-[trans-4-(3-Methyl-6-oxo-2,7-dihydropyrazolo[3,4-b]pyridin-4-yl)cyclohexyl]acetic acid (**78**).* To a stirred solution of 2-[*trans*-4-(3-methyl-6-oxo-2,7-dihydropyrazolo[3,4-*b*]pyridin-4-yl)cyclohexyl]acetonitrile **21** (450 mg, 1.66 mmol) in 1,4-dioxane (4.5 mL) was added conc. HCl (1 mL). The resulting reaction mixture was stirred at 100 °C for 24 h. The mixture was concentrated under reduced pressure. The residue was diluted with water (5 mL) and the resulting precipitate was filtered, washed with water and dried under vacuum to afford 2-[*trans*-4-(3-methyl-6-oxo-2,7-dihydropyrazolo[3,4-b]pyridin-4-yl)cyclohexyl]acetic acid **78** (400 mg, 83%) as a pale brown solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.00 (br s, 3H), 5.80 (s, 1H), 2.71 (t, *J*=11.6 Hz, 1H), 2.45 (s, 3H), 2.15 (d, *J* = 6.8 Hz, 2H), 1.85 (d, *J* = 10.4 Hz, 4H), 1.75 - 1.73 (m, 1H), 1.40 (q, *J* = 12.8 Hz, 2H), 1.16 (q, *J* = 11.2 Hz, 2H).

[(1S,2R*)-2-methylcyclohexyl]methanol (**80**).* To a solution of methyl (1*S**,2*R**)-2-methylcyclohexanecarboxylate **79** [1] (3.00 g, 19.2 mmol) in THF (30 mL) was added 1 M $LiAlH_4$ in THF (38.4 mL, 38.4 mmol) slowly at −30 °C. The resulting mixture was stirred at −30 °C for 2 h and was then warmed to 0 °C. The reaction was quenched with NH_4Cl (sat) and the mixture was extracted with diethyl ether (2 × 100 mL). The combined organic layers were dried over Na_2SO_4, concentrated under reduced pressure and the residue was purified by column chromatography (silica gel column, DCM) to afford [(1*S**,2*R**)-2-methylcyclohexyl]methanol **80** (2.04 g, 83%) as a light yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 3.56 – 3.44 (m, 2H), 2.39 – 2.37 (m, 3H), 1.95 (m, 1H), 1.65 (m, 2H), 1.46 – 1.36 (m, 6H), 0.80 (m, 3H).

(1S*,2R*)-2-methylcyclohexanecarbaldehyde (59d). To a solution of [(1*S**,2*R**)-2-methylcyclohexyl]methanol **80** (2.04 g, 15.9 mmol) in DCM (60 mL) was added $NaHCO_3$ (1.31g, 15.9 mmol) followed by Dess-Martin periodinane (9.94 g, 23.4 mmol) slowly at 0 °C. The resulting mixture was stirred at rt for 3 h. On completion, the reaction mixture was diluted with DCM (100 mL) and filtered through celite. The filtrate was concentrated under reduced pressure and the residue was purified by column chromatography (silica gel column, petroleum ether:EtOAc 90:10) to afford (1*S**,2*R**)-2-methylcyclohexanecarbaldehyde **59d** (0.600 g, 30%) as a light yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 9.85 (s, 1H), 2.40 (m, 1H), 2.21 (m, 1H), 1.85 (m, 1H), 1.79 – 1.45 (m, 6H), 1.35 (m, 1H), 0.90 (m, 3H).

*Isopropyl 5-norbornene-2-exo-carboxylate (**82**) and isopropyl 5-norbornene-2-endo-carboxylate (**83**).* Adapted from Ricks et al.[31] To a solution of 5-norbornene-2-carboxylic acid **81** (commercial grade, *endo*/*exo* mixture, 1.38 g, 10.0 mmol) in 1,2-dichloroethane (10 mL) was added DMAP (367 mg, 3.00 mmol), 2-propanol (0.91 mL, 12 mmol) and EDCI (2.30 g, 12.0 mmol). The resulting white suspension was stirred at rt (slightly exothermic) for 2 h at which point a clear solution was obtained and TLC showed complete consumption of the starting material. The solution was poured into water/sat. $NaHCO_3$ solution 1:1 (70 ml) and the mixture was extracted with DCM (2 × 80 mL). The combined organic phases were washed with brine (70 mL), dried over Na_2SO_4, and volatiles were evaporated to afford a turbid, pale yellow oil. The *endo* and *exo* isomers were separated by chromatography in two stages. First, the crude product was purified using a Grace Reveleris system (silica gel column, heptane:EtOAc 100:0 → 90:10) to afford one fraction containing a ~4:1 *endo*/*exo* mixture (1.38 g, colorless liquid) followed by a second fraction containing the pure *endo* isomer **83** (311 mg). The first fraction was then purified again by column chromatography (silica gel column, heptane:diisopropyl ether 98:2) to afford one fraction containing the pure *exo* isomer **82** (353 mg, 14%, colorless liquid, contains ~25% heptane), followed by a second fraction containing the pure *endo* isomer **83** (878 mg). This was combined with the pure *endo* fraction from the first chromatographic separation to afford the pure *endo* isomer **83** (1.05 g, 55%).

Isopropyl 5-norbornene-2-*exo*-carboxylate **82**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.16 – 6.07 (m, 2H), 5.01 (hept, *J* = 6.3 Hz, 1H), 3.02 (br s, 1H), 2.91 (br s, 1H), 2.22 – 2.14 (m, 1H), 1.96 – 1.86 (m, 1H), 1.56 – 1.49 (m, 1H), 1.42 – 1.29 (m, 2H), 1.24 (dd, *J* = 6.3, 1.0 Hz, 6H).

Isopropyl 5-norbornene-2-*endo*-carboxylate **83**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.18 (dd, *J* = 5.7, 3.0 Hz, 1H), 5.92 (dd, *J* = 5.7, 2.8 Hz, 1H), 4.94 (hept, *J* = 6.3 Hz, 1H), 3.20 (s, 1H), 2.96 – 2.84 (m, 2H), 1.94 – 1.81 (m, 1H), 1.48 – 1.37 (m, 2H), 1.34 – 1.23 (m, 2H), 1.20 (dd, *J* = 6.3, 1.8 Hz, 6H).

*5-Norbornene-2-endo-methanol (**84**).* Adapted from Ricks et al.[31] To a solution of isopropyl 5-norbornene-2-*endo*-carboxylate (500 mg, 2.77 mmol) in THF (6 mL) was added 1 M $LiAlH_4$ solution in THF (1.75 mL, 1.75 mmol) over 2 min at rt. The resulting pale yellow solution was stirred for 1 h at 40 °C and was then cooled to rt. The reaction was quenched by the sequential addition of water (70 µL), 15% aq. NaOH (70 µL) and water (210 µL). The solid precipitate formed was removed by filtration and the filter cake was washed with diethyl ether (30 mL). The filtrate was washed with brine (30 mL) and the aqueous layer was extracted with diethyl ether (30 mL). The combined organic phases were dried over Na_2SO_4 and volatiles were evaporated to afford 5-norbornene-2-*endo*-methanol **84** as a colorless oil (408 mg) that was used without purification.

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.15 (dd, *J* = 5.8, 3.0 Hz, 1H), 5.96 (dd, *J* = 5.8, 2.9 Hz, 1H), 3.40 (dd, *J* = 10.3, 6.5 Hz, 1H), 3.26 (dd, *J* = 10.5, 8.8 Hz, 1H), 2.93 (br s, 1H), 2.81 (br s, 1H), 2.37 – 2.22 (m, 1H), 1.82 (ddd, *J* = 11.6, 9.2, 3.8 Hz, 1H), 1.50 – 1.41 (m, 1H), 1.30 – 1.22 (m, 1H), 0.53 (ddd, *J* = 11.6, 4.5, 2.6 Hz, 1H).

*Norbornane-2-endo-methanol (**85**).* To a solution 5-norbornene-2-*endo*-methanol **84** (~90% purity, 185 mg, 1.34 mmol) in EtOAc (4.6 mL) was added 10% Pd/C (18.5 mg, 0.0174 mmol). The reaction was hydrogenated for 4 h 10 min at rt and ambient pressure (complete conversion by TLC). The catalyst was removed by filtration and the filtrate was evaporated to afford norbornane-2-*endo*-methanol **85** (~90% purity, 181 mg, 96%) as a colorless oil that was used without purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.70 – 3.43 (m, 2H), 2.27 (br s, 1H), 2.20 (br s, 1H), 2.14 – 1.98 (m, 1H), 1.72 (ddd, *J* = 12.0, 4.7, 2.9 Hz, 1H), 1.61 – 1.44 (m, 2H), 1.41 – 1.24 (m, 3H), 1.14 – 1.01 (m, 1H), 0.63 (ddd, *J* = 12.2, 5.2, 2.2 Hz, 1H).

*Norbornane-2-endo-carbaldehyde (**59f**).* To a solution of oxalyl chloride (0.16 mL, 1.9 mmol) in DCM (8.5 mL) at −75 °C was added a solution of DMSO (0.183 mL, 2.58 mmol) in DCM (0.45 mL) via syringe over 4 min while maintaining the temperature between −75 °C and −70 °C. The resulting colorless solution was stirred for 10 min at −75 °C. Then a solution of norbornane-2-*endo*-methanol **85** (163 mg, 1.29 mmol) in DCM (1.5 mL) was added via syringe over 3 min while maintaining the temperature between −75 °C and −70 °C. After 30 min at −75 °C, triethylamine (0.600 mL, 4.30 mmol) was added via syringe over 3 min at −75 °C to −68 °C. The viscous colorless solution was stirred for a further 20 min at −75 °C. The cooling bath was then removed, and the mixture was warmed to rt over 30 min. After a further 30 min at rt, the resulting white turbid mixture was washed with water (30 ml). The aqueous phase was extracted with DCM (30 mL) and the combined organic phases were washed with brine, dried over Na_2SO_4, and concentrated under evaporation at max. 30 °C / 50 mbar to afford a yellow turbid oil. The crude product was purified by column chromatography (silica gel column, petroleum ether:TBME 99:1 → 98:2) to afford norbornane-2-*endo*-carbaldehyde (115 mg, 68%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.77 (*d*, J = 1.1 Hz, 1H), 2.77 – 2.66 (m, 2H), 2.31 (br t, 1H), 1.73 – 1.47 (m, 3H), 1.47 – 1.33 (m, 2H), 1.32 – 1.18 (m, 2H), 0.93 – 0.80 (m, 1H).

*Norbornane-2-exo-carbaldehyde (**59e**).* Prepared using the method described above for the *endo* isomer **59f**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.63 (d, *J* = 1.5 Hz, 1H), 2.61 – 2.53 (m, 1H), 2.38 – 2.27 (m, 2H), 1.95 – 1.84 (m, 1H), 1.69 – 1.47 (m, 3H), 1.40 – 1.19 (m, 3H), 0.94 – 0.78 (m, 1H).

*Methyl trans-4-(methylsulfonyloxymethyl)cyclohexane-carboxylate (**89**).* To a solution of methyl *trans*-4-(hydroxymethyl)cyclohexanecarboxylate **88** (836 mg, 4.85 mmol) in DCM (10 mL) was added Et_3N (1.01 mL, 7.28 mmol). The solution was cooled in an ice bath and MsCl (451 µL, 5.83 mmol) was added dropwise over 5 min. The mixture was stirred at 0 °C for 20 min after which the reaction was quenched by the addition of water (20 mL), 4 N HCl (1.8 mL, 7.3 mmol) and DCM (20 mL). The layers were separated, the organic layer was washed with sat. $NaHCO_3$, dried over $MgSO_4$, filtered and evaporated to afford methyl *trans*-4-(methylsulfonyloxymethyl)cyclo-hexanecarboxylate **89** (1.21 g, 99%) which was used without purification. ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.01 (d, *J* = 6.2 Hz, 2H), 3.59 (s, 3H), 3.15 (s, 3H), 2.25 (tt, *J* = 12.2, 3.6 Hz, 1H), 2.00 – 1.87 (m, 2H), 1.85 – 1.72 (m, 2H), 1.72 – 1.56 (m, 1H), 1.43 – 1.25 (m, 2H), 1.21 – 0.95 (m, 2H).

*Methyl trans-4-(cyanomethyl)cyclohexanecarboxylate (**90**).* A solution of methyl *trans*-4-(methylsulfonyloxymethyl)cyclohexanecarboxylate **89** (1.22 g, 4.87 mmol) in DMSO (2 mL) was added to a suspension of KCN (635 mg, 9.75 mmol) in DMSO (2 mL) and the mixture was stirred at rt for 30 min. The mixture was diluted with water (20 mL) and sat. $NaHCO_3$ (3 mL). It was extracted with ether (3 × 20 mL), the combined extracts were washed with brine, dried over $MgSO_4$, filtered and evaporated to dryness to afford methyl *trans*-4-(cyanomethyl)cyclohexanecarboxylate **90** (720 mg, 81%) which was used without purification. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.58 (s, 3H), 2.44 (d, *J* = 6.5 Hz, 2H), 2.24 (tt, *J* = 12.2, 3.6 Hz, 1H), 1.98 – 1.85 (m, 2H), 1.84 – 1.71 (m, 2H), 1.69 – 1.49 (m, 1H), 1.44 – 1.25 (m, 2H), 1.17 – 0.97 (m, 2H).

*2-[trans-4-(Hydroxymethyl)cyclohexyl]acetonitrile (**91**).* To a solution of methyl *trans*-4-(cyanomethyl)cyclohexanecarboxylate **90** (720 mg, 3.97 mmol) in dry THF (20 mL) was added 2.0 M $LiBH_4$ solution in THF (4.0 mL, 8.0 mmol) and the resulting colorless solution was stirred at rt for 2 h and then at 40 °C overnight. The reaction was quenched by the addition of brine (30 mL) and extracted with EtOAc (3 × 30 mL). The organic phase was dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford 2-[*trans*-4-(hydroxymethyl)cyclohexyl]acetonitrile **91** (571 mg, 84%) as a clear oil which was used without purification. ^{1}H NMR (600 MHz, DMSO-d_6) δ 4.36 (t, *J* = 5.3 Hz, 1H), 3.22 – 3.17 (m, 2H), 2.42 (d, *J* = 6.5 Hz, 2H), 1.81 – 1.70 (m, 4H), 1.57 – 1.47 (m, 1H), 1.32 – 1.22 (m, 1H), 1.06 – 0.97 (m, 2H), 0.94 – 0.82 (m, 2H).

*2-(trans-4-Formylcyclohexyl)acetonitrile (**59j**)*. A solution of oxalyl chloride (0.47 mL, 5.6 mmol) in DCM (25 mL) under Ar was cooled to −70 °C. A solution of DMSO (529 µL, 7.45 mmol) in DCM (2 mL) was added slowly over 5 min, such that the temperature was kept between −65 and −70 °C. The reaction mixture was stirred at −70 °C for 15 min. A solution of 2-[*trans*-4-(hydroxymethyl)cyclohexyl]-acetonitrile **91** (571 mg, 3.73 mmol) in DCM (5 mL) was added slowly, such that the temperature was kept between −75 and −70 °C. The reaction mixture was stirred at −75 °C for 30 min after which Et_3N (1.73 mL, 12.4 mmol) was added dropwise, such that the temperature was kept between −74 °C and −68 °C. The resulting solution was stirred at −75 °C for 30 min. The cooling bath was removed and the temperature was raised to room temperature over 30 min. After a further 5 min at room temperature, the clear yellow solution was quenched with 150 ml water. The phases were separated and the aqueous phase was extracted with DCM (2 × 100 mL). The combined organic phases were washed with brine, dried over Na_2SO_4, filtered and concentrated by evaporation to afford 2-(*trans*-4-

formylcyclohexyl)acetonitrile **59j** (500 mg, 75%) as a clear oil which was used without purification.

*Benzyl 5-norbornene-2-carboxylate (**92** and **93**).* Adapted from Ricks et al.[31] To a solution of 5-norbornene-2-carboxylic acid **81** (commercial grade, *endo*/*exo* mixture, 20 g, 50.0 mmol) in DCM (600 mL) were added benzyl alcohol (18 mL, 28.5 mmol), DCC (31.6 g, 53.0 mmol) and DMAP (17.6 g, 50.0 mmol), and the resulting mixture was stirred at rt for 16 h. The reaction mixture was washed with 1 N NaOH followed by 1 N HCl. The organic layer was dried over anhydrous Na_2SO_4, concentrated under reduced pressure, and the residue was purified by column chromatography (silica gel column, petroleum ether:EtOAc 98:2) to afford benzyl 5-norbornene-2-*endo*-carboxylate **92** (10 g, 26%) and benzyl 5-norbornene-2-*exo*-carboxylate **93** (2 g, 10%) as a light yellow oils.

Benzyl 5-norbornene-2-*endo*-carboxylate **92**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 – 7.30 (m, 5H), 6.16 (d, J = 4.0 Hz, 1H), 5.82 (d, J = 4.0 Hz, 1H), 5.04 (s, 2H), 3.32 (s, 1H), 3.14 – 3.06 (m, 2H), 2.86 (s, 1H), 1.90 – 1.89 (m, 1H), 1.31 – 1.27 (m, 2H).

Benzyl 5-norbornene-2-*exo*-carboxylate **93**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 – 7.30 (m, 5H), 6.18 – 6.15 (m, 2H), 5.04 (s, 2H), 3.15 (s, 1H), 2.90 (m, 2H), 2.15 (m, 1H), 1.95 (m, 1H), 1.56 (m, 1H), 1.45 (m, 1H).

Benzyl (1S,2R*,4S*,5R*)-5-hydroxynorbornane-2-carboxylate (**94**) and benzyl (1R*,2R*,4S*,6S*)-6-hydroxynorbornane-2-carboxylate (**95**).* Adapted from Chen et al.[32] To a solution of benzyl 5-norbornene-2-*exo*-carboxylate **93** (15 g, 65.7 mmol) in THF (150 mL) was added 1 M $BH_3 \cdot$ THF (263 mL, 263 mmol) slowly at −5 °C under argon. The mixture was stirred at −5 °C for 1 h, after which 1 M KH_2PO_4 (200 mL) and 30% H_2O_2 (300 mL) were added sequentially. The resulting mixture was stirred at −5 °C for 2h. The reaction mixture was diluted with brine and extracted with EtOAc (2 × 500 mL). The combined organic layers were dried over Na_2SO_4, concentrated under reduced pressure, and the residue was purified by column chromatography (silica gel column, petroleum ether:EtOAc 70:30) to afford benzyl (1*S**,2*R**,4*S**,5*R**)-5-hydroxynorbornane-2-carboxylate **94** (4 g, 25%) and benzyl (1*R**,2*R**,4*S**,6*S**)-6-hydroxynorbornane-2-carboxylate **95** (4 g, 25%) as light yellow oils.

Benzyl (1*S**,2*R**,4*S**,5*R**)-5-hydroxynorbornane-2-carboxylate **94:** ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40 – 7.27 (m, 5H), 5.10 (s, 2H), 3.85 – 3.76 (m, 1H), 2.60 – 2.55 (m, 1H), 2.32 – 2.20 (m, 2H), 1.95 – 1.84 (m, 1H), 1.81 – 1.66 (m, 1H), 1.63 – 1.53 (m, 1H) 1.51 – 1.16 (m, 3H).

Benzyl (1*R**,2*R**,4*S**,6*S**)-6-hydroxynorbornane-2-carboxylate **95:** ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35 (s, 5H), 5.11 (d, J = 1.5 Hz, 2H), 3.83 (br d, J = 6.9 Hz, 1H), 2.45 (s, 1 H), 2.34 (br s, 1H), 2.22 (dd, J = 9.0, 5.7 Hz, 1H) 1.85 – 1.76 (m, 1H), 1.72 – 1.63 (m, 1H), 1.60 – 1.53 (m, 1H), 1.42 – 1.24 (m, 3H).

Benzyl (1S,2R*,4S*)-5-oxonorbornane-2-carboxylate (**96**).* To a solution of benzyl (1*S**,2*R**,4*S**,5*R**)-5-hydroxynorbornane-2-carboxylate **94** (3.8 g, 15.4 mmol) in DCM (80 mL) was added Dess-Martin periodinane (6.54 g, 15.4 mmol) at 0 °C, and the mixture was stirred at rt for 3 h. The reaction mixture was diluted with DCM (100 mL), filtered through a celite pad, and the filtrate was concentrated under reduced pressure. The residue was purified by column chromatography (silica gel column, petroleum ether:EtOAc 80:20) to afford benzyl (1*S**,2*R**,4*S**)-5-oxonorbornane-2-carboxylate **96** (2 g, 54%) as a light yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40 – 7.32 (m, 5H), 5.15 (s, 2H), 2.97 – 2.91 (m, 1H), 2.70 – 2.59 (m, 2H), 2.29 – 2.09 (m, 2H), 1.98 – 1.81 (m, 2H), 1.76 – 1.65 (m, 2H).

Benzyl (1S,2R*,4S*)-5-(cyanomethylene)norbornane-2-carboxylate (**97**).* To a solution of potassium *tert*-butoxide (1.37 g, 12.3 mmol) in THF was added diethyl cyanomethylphosphonate (1.98 g, 12.3 mmol) at 0 °C. The resulting solution was stirred at rt for 1h after which benzyl (1*S**,2*R**,4*S**)-5-oxonorbornane-2-carboxylate **96** (2 g, 8.19 mmol) was added, and stirring was continued for 3 h at rt. The reaction mixture was diluted with water (50 mL) and extracted with EtOAc (2 × 50 mL). The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography (silica gel column, petroleum ether:EtOAc 70:30) to afford benzyl (1*S**,2*R**,4*S**)-5-(cyanomethylene)norbornane-2-carboxylate **97** (1.5 g, 68%) as a viscous oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36 (s, 5H), 5.13 (s, 2H), 5.35 – 5.05 (m, 1H), 3.35 (br s, 0.5H), 2.97 (br d, J = 2.9 Hz, 0.5H), 2.87 – 2.73 (m, 1H), 2.47 (br dd, J = 13.9, 4.8 Hz, 1H), 2.17 (br s, 2H), 1.75 – 1.56 (m, 2H), 1.45 (br d, J = 10.2 Hz, 2H).

2-[(1S,4S*,5R*)-5-(Hydroxymethyl)norbornan-2-ylidene]acetonitrile (**98**).* To a solution of benzyl (1*S**,2*R**,4*S**)-5-(cyanomethylene)norbornane-2-carboxylate **97** (1.5 g, 5.61 mmol) in THF (30 mL) was added $LiBH_4$ (0.353 g, 16.85 mmol) at 0 °C, and the resulting mixture was stirred at rt for 16 h. The reaction was quenched with sat. NH_4Cl at 0 °C and extracted with EtOAc (2 × 50 mL). The combined organic layers were dried over Na_2SO_4, concentrated under reduced pressure, and the residue was purified by column chromatography (silica gel column, petroleum ether:EtOAc 60:40) to afford 2-[(1*S**,4*S**,5*R**)-5-(hydroxymethyl)norbornan-2-ylidene]acetonitrile **98** (0.8 g, 87%) as a light yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.37 – 5.20 (m, 1H), 3.72 – 3.42 (m, 2H), 3.31 – 3.26 (m, 0.5H), 2.90 (br d, J = 3.9 Hz, 0.5H), 2.54 – 2.46 (m, 2H), 2.29 – 2.21 (m, 1H), 2.15 – 2.05 (m, 1H), 1.82 – 1.71 (m, 1H), 1.60 – 1.45 (m, 1H), 1.40 (br dd, J = 10.3, 1.5 Hz, 1H), 1.35 – 1.26 (m, 1H).

2-[(1S,4S*,5R*)-5-Formylnorbornan-2-ylidene]acetonitrile (**59o**).* To a solution of 2-[(1*S**,4*S**,5*R**)-5-(hydroxymethyl)norbornan-2-ylidene]acetonitrile **98** (0.800 g, 4.90 mmol) in DCM (30 mL) was added Dess-Martin periodinane (3.12 g, 7.36 mmol) at 0 °C, and the mixture was stirred at rt for 3 h. The reaction mixture was diluted with DCM (100 mL), filtered through a celite pad, and the filtrate was concentrated under reduced pressure. The residue was purified by column chromatography (silica gel column, petroleum ether:EtOAc 90:10) to afford 2-[(1*S**,4*S**,5*R**)-5-formylnorbornan-2-ylidene]acetonitrile **59o** (0.750 g, 94%) as a light yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.72 (d, J = 6.9 Hz, 1H), 5.08 (s, 1H), 3.40 (br d, J = 3.9 Hz, 1H), 3.09 – 2.95 (m, 1H), 2.87 (br dd, J = 15.9, 3.7

Hz, 2H), 2.61 – 2.30 (m, 2H), 2.27 – 2.13 (m, 1H), 1.65 – 1.50 (m, 2H).

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Molecular formula strings (CSV)

General biology experimental procedures, NMR spectra for the structure elucidation of compound **50** and determination of the relative stereochemistry of compounds **39**-**42**, UPLC chromatograms of compounds **21**, **27** and **40**, and details of the kinase panel screen (PDF).

Accession Codes

PDB codes are the following: 6TPD for **6**; 6TPE for **21**; 6TPF for **27**. The authors will release the atomic coordinates and experimental data upon article publication.

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Author Contributions

T.H.J., M.L., M.N.B., J.L., J.G.S., and A.R. contributed to the design of compounds, computer modelling, and interpretation of results. B.B.H., T.H.J., M.L., R.S., T.V., S.R., and V.R.N. designed the synthetic routes and synthesized compounds. M.A.C. performed the pharmacokinetic studies. A.J. and C.M. performed the enzymatic and cell-based JAK assays. A.R. and P.L. wrote the manuscript.

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ABBREVIATIONS

Aq. sol., aqueous solubility; Cpd, compound; *c*-Pr, cyclopropyl; DIPEA, *N*,*N*-diisopropylethylamine; DFT, Density functional theory; HATU, 1-[*bis*(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate; HLM, human liver microsomes; JAK, Janus kinase; LLE, ligand lipophilic efficiency; SEM, standard error of the mean; SPR, surface plasmon resonance; STAT, signal transducer and activator of transcription; TR-FRET, time-resolved Förster resonance energy transfer; TYK2, tyrosine kinase 2; UPLC, ultra performance liquid chromatography.

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