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Design and synthesis of tricyclic cores for kinase inhibition

Stacy Van Epps [a,*], Bryan Fiamengo [a,*], Jeremy Edmunds [a], Anna Ericsson [d], Kristine Frank [b], Michael Friedman [a], Dawn George [a], Jonathan George [a], Eric Goedken [a], Brian Kotecki [c], Gloria Martinez [a], Philip Merta [b], Michael Morytko [a], Shashank Shekhar [c], Barbara Skinner [e], Kent Stewart [b], Jeffrey Voss [a], Grier Wallace [a], Lu Wang [a], Lu Wang [a], Neil Wishart [a,*]

[a] Abbott Bioresearch Center, 381 Plantation St, Worcester, MA 01605, USA
[b] Abbott Laboratories, 100 Abbott Park Rd, Abbott Park, IL 60064, USA
[c] Abbott Laboratories, 1401 Sheridan Rd, North Chicago, IL 60064, USA
[d] Forma Therapeutics, 500 Arsenal St, Watertown, MA 02472, USA
[e] Abbott Laboratories, 381 Plantation St. Worcester MA, USA

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ABSTRACT

Interest in therapeutic kinase inhibitors continues to grow beyond success in oncology. To date, ATP-mimetic kinase inhibitors have focused primarily on monocyclic and bicyclic heterocyclic cores. We sought to expand on the repertoire of potential cores for kinase inhibition by exploring tricyclic variants of classical bicyclic hinge binding motifs such as pyrrolopyridine and pyrrolopyrazine. Herein we describe the syntheses of eight alternative tricyclic cores as well as in vitro screening results for representative kinases of potential therapeutic interest.

In recent years drug discovery organizations have taken an increased interest in kinases inhibitors to treat multiple diseases beyond cancer, such as rheumatoid arthritis (RA), psoriasis, inflammatory bowel disease, and other autoimmune diseases.[1,2] Recent clinical results from Pfizer's Jak inhibitor tofacitinib[3] and AstraZeneca and Rigel's kinase inhibitor fostamatinib[4] (Fig. 1) indicate that kinase inhibitors can demonstrate clinical efficacy as well as drug tolerability in chronic inflammatory indications.

The majority of the kinase inhibitors identified to date bind to the ATP binding site in the active conformation, and are typically referred to as type 1 inhibitors. As a result of extensive activities in this area of research it is becoming increasingly more challenging to identify novel type 1 chemotypes as starting points for small molecule kinase drug discovery programs. Limited by the specificities of the ATP pharmacophore, particularly the ability to interact with the hinge region of the kinase, as well as the need to maintain drug-like properties within the molecule, a variety of monocyclic and bicyclic heterocyclic hinge binders have been developed.[5] Given the expansion of prior art around such chemotypes, we investigated the ability to modify commonly adopted kinase hinge binders, such as 1*H*-pyrrolo[2,3-*d*]pyridine and 5*H*-pyrrolo[2,3-*b*]pyrazine, to identify several novel tricyclic heterocycles (Fig. 2). Additionally, by fusing a third ring to such kinase hinge binding cores, we are able to incorporate conformationally preferred substituents that are able to interact in a complementary manner to the protein target.

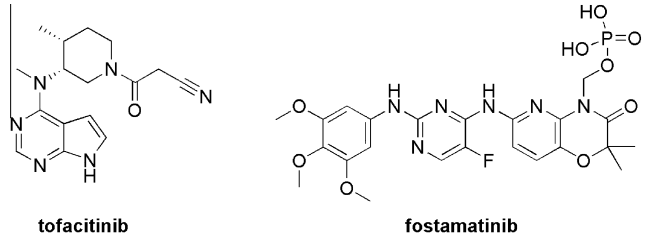

Figure 1. Recent non-oncology kinase inhibitors in the clinic.

Figure 2. Location of third ring.

* Corresponding authors.
E-mail address: stacy.vanepps@abbott.com (S. Van Epps).

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Herein we describe the synthesis of eight different tricyclic cores starting from commercially available heterocycles. Detailed experimental procedures for the synthesis of such cores have previously been described.[6] Our interests focused on the synthesis of unique tricyclic pyrrolopyridines and pyrrolopyrazines. All cores were initially synthesized with an unadorned cyclohexyl substituent. This was done in order to simplify the initial chemistry efforts and to allow for ready comparisons across the series. Starting with tricyclic variants of pyrrolopyridines the synthetic route to 3,6-dihydropyrazolo[4,3-*d*]pyrrole[2,3-*b*]pyridines **5** and **6** as well as 6*H*-isoxazolo[4,5-*d*]pyrrole[2,3-*b*]pyridine **8** is presented in Scheme 1. Treatment of commercially available aldehyde **1** with cyclohexylmagnesium chloride and subsequent oxidation of the alcohol afforded ketone **2**. Hydrazone formation using either hydrazine or methyl-hydrazine followed by cyclization gave final compounds **5** and **6**, respectively.[7] Oxime formation from **2** using hydroxylamine provided intermediate **7** which was cyclized in the presence of potassium *tert*-butoxide to afford tricycle **8**.

The synthesis of 1,6-dihydroimidazo[4,5-*d*]pyrrolo[2,3-*b*]pyridine cores (**15, 23, 24**) are exemplified in Schemes 2 and 3. Nitration of 4-Chloro-3-iodopyridin-2-amine **9** with potassium nitrate resulted in **10** (Scheme 2). Sonogashira cross coupling of **10** with TMS-acetylene followed by desilylation and modified rhodium-catalyzed Larock cyclization led to common intermediate **12**.[8] S_NAr displacement of chloride **12** with cyclohexylamine gave **13**, which underwent tin chloride-promoted reduction to diamine **14**. Subsequent ring closure utilizing triethyl orthoformate generated the desired product **15** in low yield. Further investigation of additional analogs in this series led to protection of the pyrrole nitrogen as a means to optimize reaction yields (Scheme 3).

Thus azaindole **12** was tosyl-protected prior to nitro-assisted chloro displacement with cyclohexylamine providing **17** (Scheme 3). In similar fashion, tin chloride-promoted reduction of the nitro group led to diamine **18** which readily underwent acylation to furnish **19** and **20**. Cyclization of **19** with $POCl_3$ and subsequent deprotection furnished tricycle **23**. Intramolecular dehydrative ring closure of **20** using propylphosphonic anhydride led to **22** in an improved 68% yield. Hydrolysis of the tosyl protected pyrrole provided final compound **24**. These types of tricyclic kinase inhibitors have also very recently been reported via nitration of 4-chloro-1-phenylsulfonyl-1*H*-pyrrolo[2,3-*b*]pyridine.[9,10]

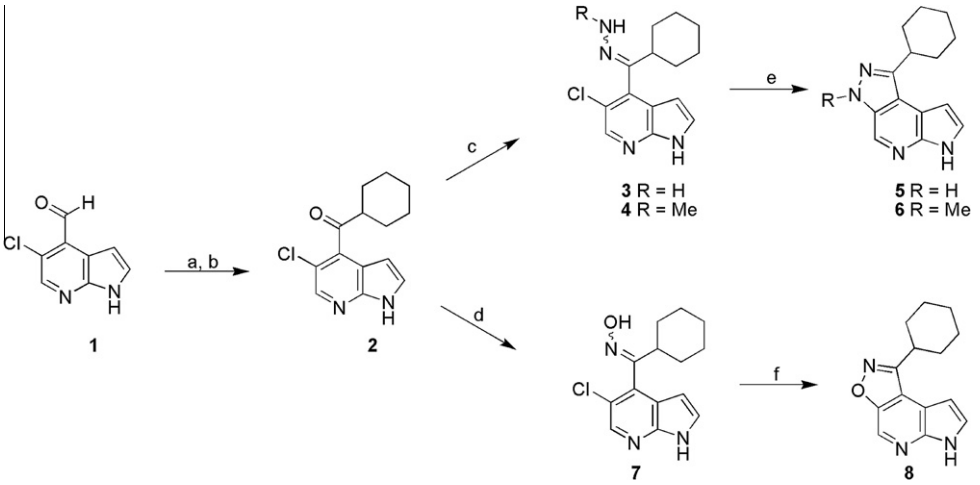
As an alternative to aromatic azaindole-containing tricycles, we expanded our focus to a tricyclic system containing a partially saturated ring (Scheme 4). Starting from commercially available pyrrolopyridine **25**, intermediate **26** was synthesized using *ortho*-lithiation followed by trapping with ethyl chlorofomate.[11] A protecting group exchange and traditional functional group modifications allowed access to key aldehyde **29**. Wittig product **30** was formed using ((1,3-dioxolan-2-yl)methyl)triphenyl-phosphonium bromide and was reduced by hydrogenation to give **31**. Acidic dioxolane deprotection and subsequent intramolecular imine formation followed by reduction yielded protected tricycle **32**. The 2,3,4,7-tetrahydro-1*H*-pyrrolo[2,3-*h*][1,6]naphthyridine analog **33** was obtained after removal of tosyl protection under basic conditions.

In addition to our efforts on azaindole-containing tricycles, additional tricycles derived from pyrrolopyrazine were explored. A linear synthetic approach to 6*H*-pyrrolo[2,3-*e*][1,2,4]triazolo[4,3-*a*]pyrazine **41** is represented in Scheme 5. Treatment of aminopyrazine **34** with NBS generated **35** which underwent Sonogashira cross coupling with TMS-acetylene. One pot cyclization and protection formed intermediate **37**. Buchwald–Hartwig cross-coupling of **37** with di-*tert*-butyl hydrazodicarboxylate yielded **38** and acidic cleavage produced hydrazine **39**. Hydrazide **40** was generated using cyclohexylcarbonyl chloride and was subsequently cyclized by treatment with thionyl chloride and deprotected to afford triazole **41**.

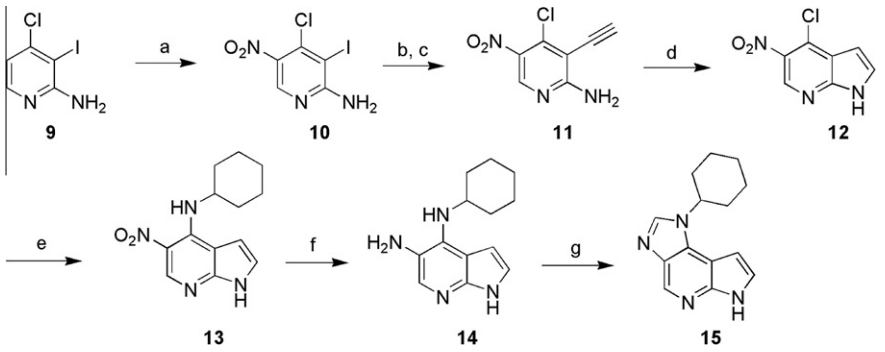
Intermediate **37** gave access to multiple tricycles: 3*H*-imidazo[1,2-*a*]pyrrole[2,3-*e*]pyrazine **44** (Scheme 6), 3*H*-dipyrrolo[1,2-*a*:2',3'-*e*]pyrazine **50** (Scheme 7), and 6*H*-imidazo[1,5-*a*]pyrrolo[2,3-*e*]pyrazine **55** (Scheme 8). As shown in Scheme 6, Buchwald-Hartwig cross-coupling of **37** with *tert*-butyl carbamate followed by alkylation and Boc deprotection afforded ketone **43**. Acidic cyclization employing 2,2,3,3,3-pentafluoropropanoic anhydride and tosyl deprotection provided the desired tricycle **44**.

Suzuki cross-coupling of key intermediate **37** with styrylboronic acid followed by oxidative cleavage using osmium tetraoxide and sodium periodate produced aldehyde **46** (Scheme 7). Horner-Wadsworth-Emmons homologation with diethyl 2-cyclohexyl-2-oxoethylphosphonate and subsequent hydrogenation led to ketone **48**. Sulfur-mediated cyclization using Belleau's reagent and tosyl hydrolysis generated final compound **50**.

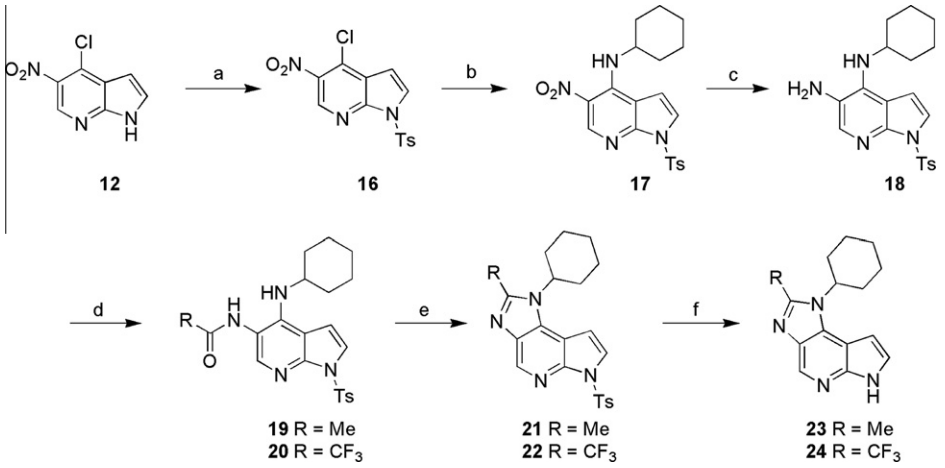
Additional utility of intermediate **37** also allowed access to the 6*H*-imidazo[1,5-*a*]pyrrolo[2,3-*e*]pyrazine core (Scheme 8). Palladium-catalyzed cyanation of **37** and subsequent hydrogenation in the presence of acid provided the HCl salt of amine **52**. Amidation



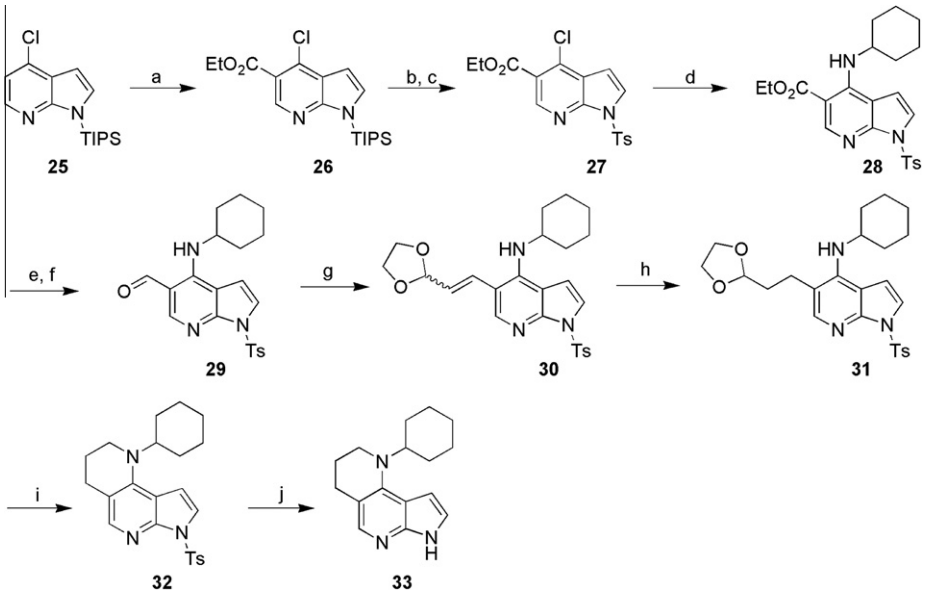
Scheme 1. Reagents and conditions: (a) cyclohexylmagnesium chloride, THF, −20 °C to rt, 16 h, 51%; (b) Dess–Martin periodinane, DCM, 0 °C, 3 h, 73%; (c) for **3**: hydrazine, AcOH, EtOH, reflux, 20 h, 48%; for **4**: methylhydrazine, AcOH, EtOH, reflux, 48 h, 72%; (d) hydroxylamine hydrochloride, AcOH, 100–130 °C, 72 h, 52%; (e) for **5**: $Pd(OAc)_2$, CyPF*t*-Bu, NaO*t*-Bu, NMP, microwave 170 °C, 40 min, 33%; for **6**: $Pd(OAc)_2$, CyPF*t*-Bu, NaO*t*-Bu, NMP, microwave 160 °C, 45 min, 65%; (f) KO*t*-Bu, DMSO, microwave 125 °C, 1.5 h, 46%.



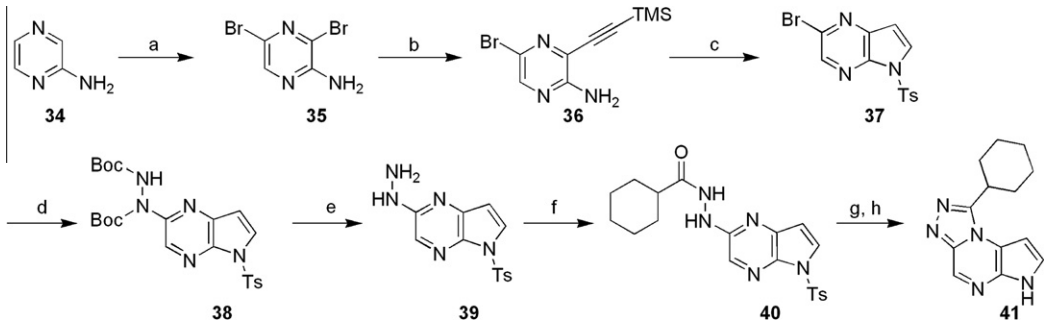
Scheme 2. Reagents and conditions: (a) KNO_3, H_2SO_4, −10 °C, 15 h, 60%; (b) TMS-acetylene, $PdCl_2(PPh_3)_2$, CuI, TEA, THF, 50 °C, 12 h, 70%; (c) KF, DMF, rt, 4 h, 87%; (d) chloro(1,5-cyclooctadiene)rhodium(I) dimer, tris(4-fluorophenyl)phosphine, DMF, 80 °C, 2 h, 81%; (e) cyclohexylamine, DMF, rt, 18 h, 82%; (f) $SnCl_2{\cdot}2H_2O$, EtOH, 55 °C, 2 h, 82%; (g) triethyl orthoformate, *p*-TsOH-H_2O, 80 °C, 5 h, 3%.



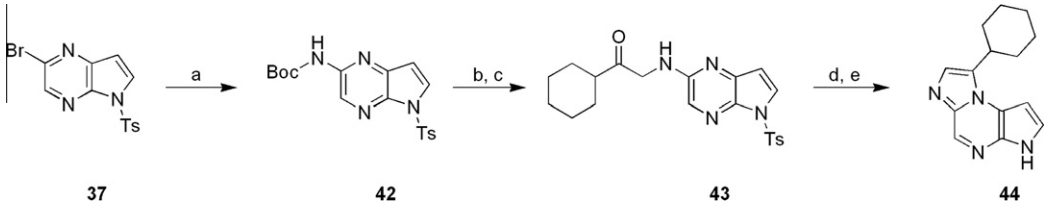
Scheme 3. Reagents and conditions: (a) NaH, TsCl, DMF, 0 °C to rt, 2 h, 98%; (b) cyclohexylamine, DIEA, DMF, rt, 30 min; (c) $SnCl_2{\cdot}2H_2O$, EtOH, 75 °C, 5 h, 79% over 2 steps; (d) for **19**: acetic anhydride, TEA, 0 °C to rt, 5 h; for **20**: trifluoroacetic anhydride, TEA, DCM, rt, 10 min, 75%; (e) for **21**: $POCl_3$, 80 °C, 16 h; for **22**: propylphosphonic anhydride, DMF, 100 °C, 16 h, 68%; (f) for **23**: 1 M aq NaOH, dioxane, 90 °C, 4 h, 6% over 3 steps; for **24**: 1 M aq NaOH, dioxane, 80 °C, 1 h, 29%.



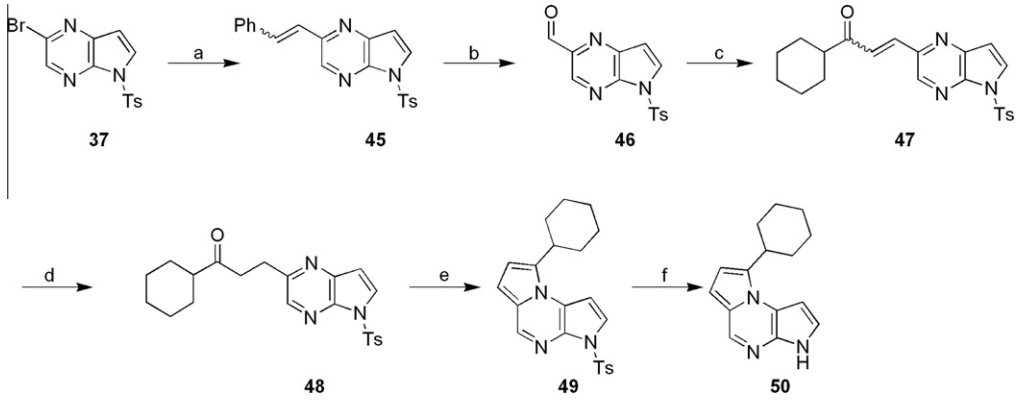
Scheme 4. Reagents and conditions: (a) *sec*-BuLi, ethyl chloroformate, THF, −78 °C to rt, 40 min, 98%; (b) TBAF, THF, 0 °C, 1 h, 52%; (c) NaH, TsCl, DMF, 0 °C to rt, 2 h, 92%; (d) cyclohexylamine, *n*-BuOH, 110 °C, 16 h, 73%; (e) DIBAL-H, toluene, -78 °C to rt, 2 h, 80%; (f) MnO_2, $CHCl_3$, rt, 16 h, 87%; (g) ((1,3-dioxolan-2-yl)methyl)triphenyl-phosphonium bromide, KO*t*-Bu, THF, 0 °C to rt, 16 h, 67%; (h) 10% Pd/C, H_2, EtOAc, rt, 1.5 h, 97%; (i) conc. HCl, EtOH, 40 °C, 2 h, then $NaBH_4$, 0 °C, 2 h, 68%; (j) 5 M aq NaOH, dioxane, 100 °C, 48 h, 60%.



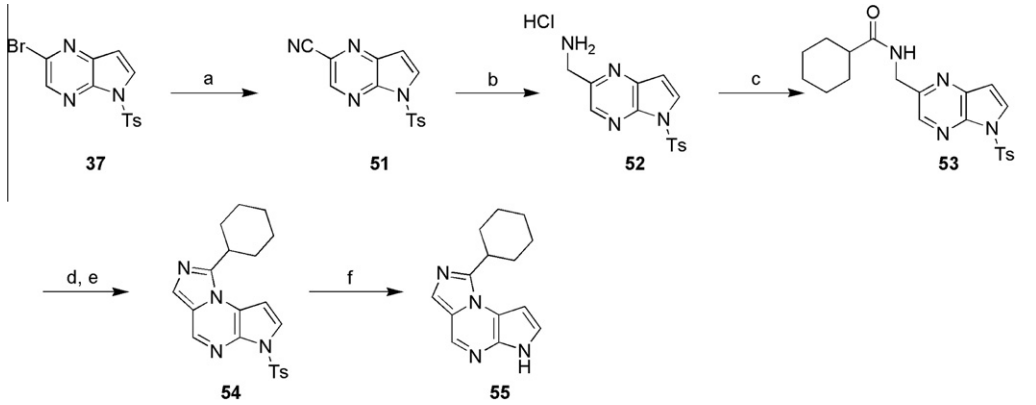
Scheme 5. Reagents and conditions: (a) NBS, Na_2CO_3, DMSO-H_2O, <15 °C to rt, 6 h, 76%; (b) TMS-acetylene, $PdCl_2(PPh_3)_2$, CuI, TEA, THF, −5 to 0 °C, 1.5 h, 88%; (c) NaH, TsCl, DMF, 0 °C to rt, 1 h, 54%; (d) di-*tert*-butyl hydrazodicarboxylate, $Pd(OAc)_2$, Xantphos, K_2CO_3, *t*-AmOH/dioxane, 95 °C, 2 h, 91%; (e) (i)H_3PO_4, THF/heptane, 70 °C, 2 h, (ii) K_2CO_3, H_2O, rt, 1 h, 94%; (f) cyclohexylcarbonyl chloride, DIEA, dioxane, 0 °C to rt, 1 h; (g) $SOCl_2$, dioxane, 90 °C, 1 h; (h) 2 M aq Na_2CO_3, MeOH, 80 °C, 72 h, 40% over 3 steps.



Scheme 6. Reagents and conditions: (a) *tert*-butyl carbamate, $Pd(OAc)_2$, Xantphos, K_2CO_3, *t*-AmOH-dioxane, 95 °C, 3 h, 74%; (b) 2-bromo-1-cyclohexylethone, NaH, DMF, 0 °C, 1 h, 83%; (c) TFA, DCM, rt, 2 h, quant.; (d) 2,2,3,3,3-pentafluoropropanoic anhydride, acetonitrile, 60 °C, 2 h, 94%; (e) 1 M aq NaOH, dioxane, 50 °C, 1 h, quant.



Scheme 7. Reagents and conditions: (a) styrylboronic acid, $PdCl_2(dppf)$–CH_2Cl_2, Na_2CO_3, THF–H_2O, 65 °C, 24 h, 92%; (b) OsO_4, $NaIO_4$, dioxane/H_2O, rt, 15 h, 92%; (c) diethyl 2-cyclohexyl-2-oxoethylphosphonate, NaH, THF, rt, 2 h, 74%; (d) 10% Pd/C, H_2, EtOAc, 1 h, quant.; (e) Belleau's reagent, THF, rt, 6 h, 42%; (f) 2 M aq NaOH, dioxane, 90 °C, 15 h, 55%.



Scheme 8. Reagents and conditions: (a) Zn, $Zn(CN)_2$, $PdCl_2(dppf)$-CH_2Cl_2, DMA, 95 °C, 16 h, 70%; (b) 5% Pd/C, H_2 40 psi, quinoline, 37% HCl, THF/H_2O, rt, 2 h, 88%; (c) cyclohexanecarbonyl chloride, Hunig's base, DCM, rt, 4 h, 80%; (d) Lawesson's reagent, dioxane, 60 °C to rt, 16 h; (e) $Hg(OAc)_2$, dioxane, rt, 30 min, 80% over 2 steps; (f) 2 M aq NaOH, dioxane, 90 °C, 5 h, 90%.

Table 1
in vitro enzyme inhibition (IC_{50})[12,13]

Compound	Aurora[b] 1 (μM)	FLT[a]-3 (μM)	Jak[c]2 (μM)	Kd[b]r (μM)	PKCα[b] (μM)	TYK2[c] (μM)
5	0.13	0.22	0.01	0.09	1.3	0.05
6	0.34	0.44	0.05	0.16	2.6	0.30
8	0.57	1.8	0.03	0.20	2.8	0.06
15	0.74[d]	na	5.7[b]	3.7 [d]	1.7	na
23	0.64 [d]	na	6.0 [b]	2.6 [d]	1.3	na
24	4.3 [d]	na	11.4 [b]	0.72 [d]	1.7	na
33	3.2	>50	na	na	na	0.53
41	15.3	27.3	0.09	2.6	18.0	1.2
44	0.04	0.24	na	1.0	na	na
50	0.03	0.15	na	0.60	na	4.8
55	0.27	0.89	na	0.91	na	na

Na = data not available.
[a] 1000 μM ATP.
[b] 100 μM ATP.
[c] 1 μM ATP.
[d] binding assay.

Table 2
in vitro Jak enzyme and cellular activity[14,15]

Compound	Jak 1 enzyme[a] (μM)	Jak 1 cell[b] (μM)	Jak 2 cell[b] (μM)	Jak2/Jak1 Cellular selectivity
5	0.003	0.17	4.2	25×
6	0.09	1.2	0.89	1×
8	0.02	0.43	1.6	4×
15	0.03	0.21	9.8	47×
23	0.02	0.18	6.6	37×
24	0.04	0.97	>20	21×
41	0.03	0.35	15.8	45×
44	0.004	0.15	4.8	32×
50	0.02	na	>20	na
55	0.01	0.37	2.8	8×

Na = data not available
[a] Enzyme inhibition (IC_{50}) at 1 μM ATP.
[b] Cellular efficacy (EC_{50}).

with cyclohexylcarbonyl chloride generated **53** which readily underwent thioamide formation using Lawesson's reagent followed by mercury catalyzed intramolecular cyclization to afford **54**. Finally, base mediated tosyl hydrolysis gave the desired tricycle **55**.

By design, the tricyclic heterocycles exemplified in (Schemes 1–8) possess hydrogen bond donor-acceptor hinge binding motifs, that have the potential to competitively bind to the ATP active site of kinases. Thus, the tricyclic inhibitors were evaluated for activity against a panel of kinases. The in vitro enzymatic assay data for a selected set of kinases for the final compounds described above are presented in Table 1. With the exception of compounds **15**, **23**, and **24** all in vitro potencies were generated in enzyme activity assays. The inhibitory activity of **15**, **23**, and **24** were determined using a trFRET binding competition assay. While these tricyclic hinge binders possess a range of binding activities and potencies against different kinases we were particularly interested by how many of them potently inhibit the Jak family kinase, Jak2. Due to our interest in exploring kinase inhibitors in non-oncology indications the analogs with activity against Jak2 were then further profiled against Jak1.

All of the compounds tested exhibited potencies <100 nM in the Jak1 enzymatic assay at an ATP concentration close to its K_m (Table 2). To facilitate a comparison of all the different tricycles, the compounds were progressed into multiple Jak cellular assays for evaluation of potency and selectivity. The majority of the compounds exhibited good inhibitory activity in the Jak1 (IL-6/pSTAT3) cellular assay. Furthermore, most of the compounds showed modest Jak2 (Epo/pSTAT5) cellular activity leading to a defined cellular selectivity window for Jak1 over Jak2. This demonstrates the possibility of identifying selective Jak1 inhibitors with compounds **15**, **23**, **41**, and **44** showing the highest level of cellular selectivity in these assays.

In conclusion, modifying the common pyrrolopyridine and pyrrolopyrazine hinge binders led to the discovery of novel tricyclic inhibitors. The heterocyclic compounds described herein exhibit a wide range of activity for various kinases including Jak kinases. To improve potency and selectivity for Jak1 over Jak2, alteration of the cyclohexyl capping group to a more optimized Jak kinase inhibitor was investigated. Subsequent reports will detail the efforts that led to potent and selective Jak1 inhibitors.

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12. In vitro kinase activity assays were performed typically at ATP concentrations near measured ATP Km values (1–1000 μM) in the presence of small biotinylated peptide substrates. Following an enzyme reaction at room temperature in 96- or 384-well plates, reactions were stopped by addition of excess EDTA and the phosphorylated product was detected by time-resolved fluorescence energy transfer (trFRET) using fluorescent (donor) antibodies that recognize the phosphorylation site on the peptide and a streptavidin-allophycocyanin (acceptor) conjugate that binds the peptide's biotinylated portion.
13. trFRET kinome profiling was performed as follows. Similar to assays first reported in Lebakken, C. S.; Riddle, S. M.; Singh, U.; Frazee, W. J.; Eliason, H. C.; Gao, Y.; Reichling, L. J.; Marks, B. D.; Vogel, K. W. *J. Biomol. Screen.*, **2009**, *8*, 924; trFRET binding assays were performed using target kinases (2-10 nM; Invitrogen), Oregon Green-labeled fluorescent probes (2× Kd of probe; range of 6.25–200 nM), terbium anti-HIS or -GST antibody (2 nM; invitrogen). The reaction buffer consists of 20 mM HEPES pH 7.4/10 mM $MgCl_2$/.0075% Triton X-100/100 μM sodium orthovanadate/1 mM dTT. Reactions are carried out in a 20 μL volume in 384-well plates, by combining kinase/probe/antibody/test compound, mixing, and equilibrating 2.5 h. Fluorescence is measured on a PerkinElmer Envision plate reader (excitation 340 nm and emission at 520/495 nm).
14. In vitro Jak1 kinase activity assay used Jakl kinase domain (aa 845–1142), biotin-TYR2 peptide (Biotin-(Ahx)-AEEEYFFLFA-amide; 2 μM) and 1 μM ATP in 50 mM MOPSO pH 6.5, 10 mM $MgC1_2$, 2 mM $MnC1_2$ (2 mM), DTT (2.5 mM), BSA (0.01% w/v), 0.1 mM Na_3VO_4 and ATP (0.001 mM). After 60 min, the enzymatic reaction was quenched by addition of EDTA (to final concentration 100 mM) and developed by addition of PT66 K (europium labeled anti-phosphotyrosine antibody cat #61T66KLB Cisbio, Bedford, MA) and 3.12 μg/mL phycolink streptavidin-allophycocyanin acceptor (cat #PJ25S, Prozyme, San Leandro, CA). Following incubation for ⩾60 min, 96-well plates were read via a Rubystar detector (BMG) using a 337 nm laser for excitation and emission wavelength of 665 nm.
15. Jak1 cellular assays (IL-6 dependent pSTAT3 in TF-1 cells) and Jak2 cellular assays (Epo-dependent pSTAT5 assays in UT-7 cells) were conducted as described in the supplemental methods in Goedken, E. R.; Devanarayan, V.; Harris, C. M.; Dowding, L. A.; Jakway, J. P.; Voss, J. W.; Wishart, N.; Jordan, D. C.; Talanian, R. V. *J. Biomol. Screen.* **2012**, *17*, 857.