Bioorganic & Medicinal Chemistry 22 (2014) 1156–1162

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Linear propargylic alcohol functionality attached to the indazole-7-carboxamide as a JAK1-specific linear probe group

Mi Kyoung Kim [†], Heerim Shin [†], Seo Young Cho, Youhoon Chong [*]

Department of Bioscience and Biotechnology, Bio/Molecular Informatics Center, Konkuk University, Hwayang-dong, Gwangjin-gu, Seoul 143-701, Republic of Korea

ARTICLE INFO

Article history:
Received 13 November 2013
Revised 4 December 2013
Accepted 7 December 2013
Available online 22 December 2013

Keywords:
Janus kinase (JAK)
Isozyme-specificity
Propargylic alcohol
Probe group

ABSTRACT

Selective inhibition of JAK1 has recently been proposed as an appropriate therapeutic rationale for the treatment of inflammatory diseases such as rheumatoid arthritis (RA). In this study, through pairwise comparison and 3D alignment of the JAK isozyme structures bound to the same inhibitor molecule, we reasoned that an alkynol functionality would serve as an isozyme-specific probe group, which would enable the resulting inhibitor to differentiate the ATP-binding site of JAK1 from those of other isozymes. The 3-alkynolyl-5-(4′-indazolyl)indazole-7-carboxamide derivatives were thus prepared, and in vitro evaluation of their inhibitory activity against the JAK isozymes revealed that the propargyl alcohol functionality endowed the 5-(4′-indazolyl)indazole-7-carboxamide scaffold with JAK1 selectivity over other JAK isozymes, particularly JAK2.

1. Introduction

The JAK/STAT pathway is the major signaling cascade in response to inflammatory and proliferative signals such as cytokine, chemokine, and growth factors. This signaling pathway consists of the Janus protein tyrosine kinase family (JAK3, JAK2, JAK1, and TYK2) and the STAT (signal transduction and transcription) family of transcription factors. Being engaged with the cytoplasmic domains of the transmembrane receptors, the JAKs become activated upon ligand-receptor binding to result in phosphorylation, dimerization, and nuclear translocation of downstream STAT proteins, which regulate transcription of STAT-dependent genes. The JAKs are coupled with specific cytokines that play essential roles in immune function,[1] inflammation,[2] and hematopoiesis.[3] For instance, signaling by the gamma common (γ_c) family of cytokines (interleukins IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21) is transmitted exclusively by JAK1 and JAK3.[4] As loss of function mutation in the γ_c chain or JAK3 results in severe combined immunodeficiency (SCID), abrogation of signaling by γ_c-cytokines and thereby inhibition of the γ_c-linked JAK3 has long been regarded as an attractive target for the treatment of immunologic disorders such as rheumatoid arthritis (RA).[2,5] Among the synthetic JAK inhibitors identified to date, Pfizer's Xelianz (Tofacitinib, CP-690-550), a pan-JAK inhibitor initially reported as a selective JAK3 inhibitor,[5b] has recently been approved by FDA for the treatment of RA.[6] However, in spite of the clinical success of Xelianz, it is unclear whether pan-JAK or isozyme-selective inhibition is required for clinical intervention of RA. In this regard, accumulating evidence suggests that, in signal transduction through γ_c-containing cytokine receptors, functional activity of JAK1 is dominant while JAK3 plays a secondary role to merely enhance the effect of JAK1.[7–11] Thus, JAK1 has become an appropriate target for immune modulation, and several JAK1-selective inhibitors have recently been identified.[12,13] In addition, ideal therapeutic agent for RA is also anticipated to have high JAK1 versus JAK2 selectivity because concurrent inhibition of JAK2 would result in significant anemia particularly dangerous for patients under immunomodulating therapy.[14]

Structure-based rational drug design, a well-known process to accelerate inhibitor lead design and optimization, has been hampered in the field of the isozyme-selective JAK kinase inhibitors because the JAK kinases share similar structure which is characterized by the presence of seven JAK homology (JH) domains. In this study, we reasoned that pairwise comparison and 3D alignment of the JAK isozyme structures bound to the same inhibitor molecule would delineate the active site variants responsible for isozyme specificity of the JAK inhibitors, which culminated in identification of the relative position of the glycine-rich loop as the key structural variants among the JAK isozymes. Herein, we report structure-based design of the JAK1-selective inhibitor, synthesis of a series of (indazolyl)indazole derivatives, and evaluation of their inhibitory activity against all JAK isozymes.

2. Results and discussion

2.1. Structure-based design of the title compounds

Three-dimensional crystal structures of the JAK isozymes bound to *C*-2 methyl imidazopyrrolopyridines (CMP) were retrieved from

* Corresponding author. Tel.: +82 220496100.
E-mail address: chongy@konkuk.ac.kr (Y. Chong).
† These two authors contributed equally on this work.

0968-0896/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmc.2013.12.033

the Protein Data Bank (PDB) [PDB ID: 3EYH (JAK1),[15] 2B7A (JAK2),[16] 3LXL (JAK3),[17] 3LXP (Tyk2)[17]], and aligned by using the 'protein structure alignment' module incorporated in the molecular modeling software Maestro. Except 3LXP, which showed significant structural difference compared with other isozymes, structures of JAK1, JAK2, and JAK3 superposed well to show only minimal deviation particularly around the ATP-binding site (Fig. 1a). Nevertheless, subtle but significant difference was observed at the so-called 'glycine loop'. Thus, due to formation of a hydrogen bond between Asp1003 and His885, the ATP-binding site of JAK1 is markedly narrow compared with JAK2 and JAK3. Moreover, Phe860 in JAK2, which corresponds to His885 in JAK1, locates its aromatic side chain away from Asp976 in the N-terminal lobe to result in relatively open ATP-binding site. Due to this interesting feature, JAK1 forms a small cavity right below the CMP-binding site, which seems to be able to accommodate short linear alkyl chain (lined with the block arrows in Fig. 1b). In addition, two polar residues, Asp1003 and His885, at the bottom of the cavity are capable of forming hydrogen bonds with the incoming inhibitor molecule.

Based on these observations, we reasoned that positioning a polar group attached to a linear tether around the His885 of JAK1 (Phe860 of JAK2) would enable the resulting inhibitor to differentiate the ATP-binding sites of JAK1 from those of other isozymes. For this purpose, propargyl alcohol ($n = 1$, Fig. 2) and but-3-yn-1-ol ($n = 2$, Fig. 2) were chosen as the isozyme-specific linear probe group, which were attached to the 3-position of the indazole-7-carbamate, a hydrogen-bonding scaffold to the hinge motif of the kinase. In order to increase binding interaction with the enzyme, additional indazole functionality was installed at the 5-position to result in the title compound, 3-alkynolyl-5-(4′-indazolyl)indazole-7-carboxamide (**1**, Fig. 2).

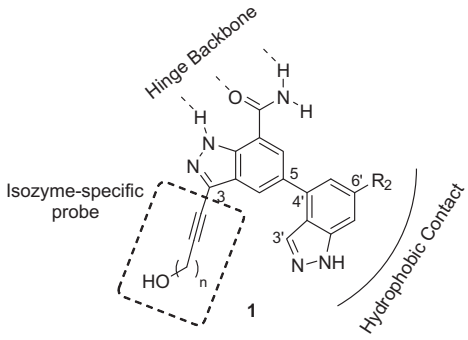


Figure 2. Structure of the designed title compound.

2.2. Syntheses of the title compounds

Syntheses of the 5-(4′-indazolyl)indazole-7-carboxamide derivatives **1a**–**1f** were performed by Suzuki coupling[18] of the 3-alkynolyl-5-bromo-indazole-7-carboxamides (**2a**–**2c**) and 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indazole derivatives (**3a**–**3b**) followed by removal of the Boc-protecting group in 35–68% combined yield (Scheme 1).

The key intermediates 3-alkynolyl-5-bromo-indazole-7-carboxamides (**2a**–**2c**) and 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indazole derivatives (**3a**–**3b**) were prepared starting from methyl 2-amino-3-methylbenzoate **4** (Scheme 2) and 2,4-dinitrotoluene **8** (Scheme 3), respectively. Thus, treatment of **4** with NBS proceeded exclusively at the *para* position of the amino functionality to give **5** in 62% yield.[19] Construction of the indazole core structure was then accomplished by acetylation followed by diazotization of **5** to give a separable mixture of **6a** (46% yield) and **6b** (35% yield).[20] The acetyl group of **6a** was removed by treatment with 6 N HCl in MeOH to give **6b** in quantitative yield. The methyl ester **6b**, thus obtained, was converted into the corresponding Boc-protected amide **2a** via hydrolysis followed by amidation and Boc-protection in 95% combined yield. Treatment of **2a** with NIS provided 3-iodoindazole **7** in 54% yield, which gave the desired indazolyl-3-alkynols **2b** and **2c** by Sonogashira coupling reactions[21] with propargyl alcohol (36% yield) and but-3-yn-1-ol (30% yield), respectively.

Similar strategy was applied to the synthesis of the 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indazole derivatives (**3a**–**3b**) (Scheme 3). Bromination of 2,4-dinitrotoluene (**8**) with NBS[22] followed by reduction of the nitro functionality with iron powder[23] provided an inseparable mixture of nitroanilines, 3-bromo-4-methyl-5-nitroaniline and 3-bromo-2-methyl-5-nitroaniline, in 3:1 ratio (74% combined yield). Boc-protection of the resulting amino functionality and reduction of the remaining nitro functionality gave a mixture of **9a** and **9b**, which were readily separated by silica gel column chromatography. The desired regioisomer **9a** underwent cyclization to the indazole core structure

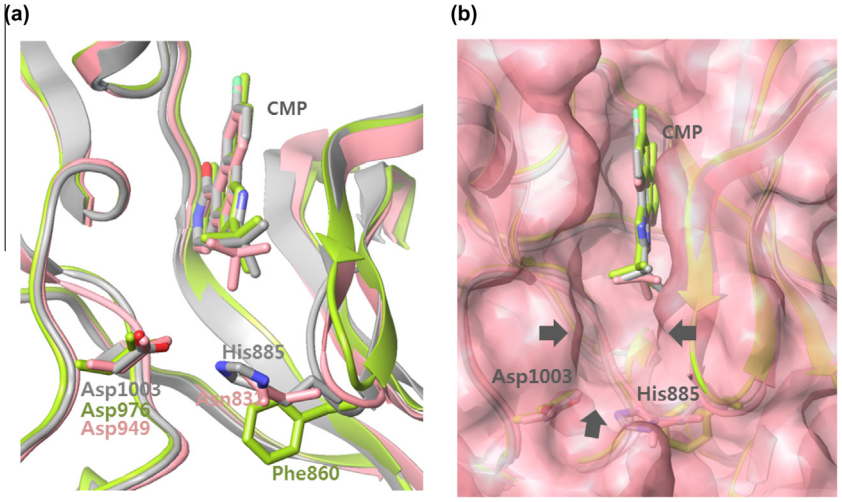
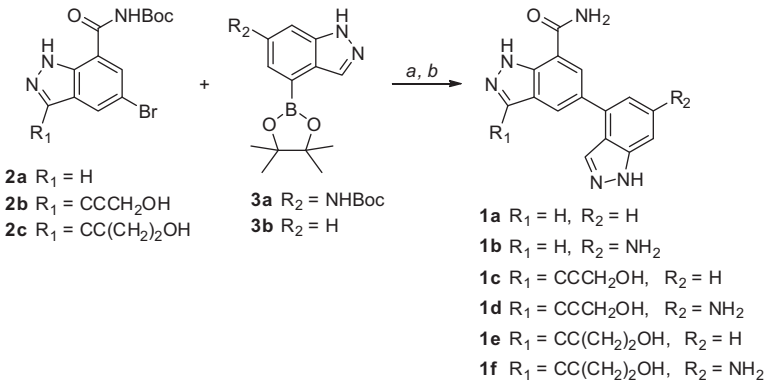
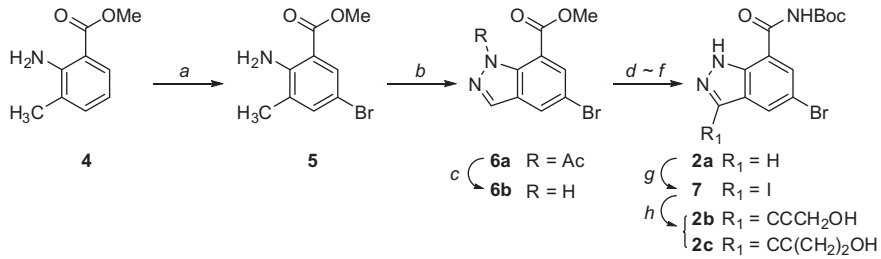


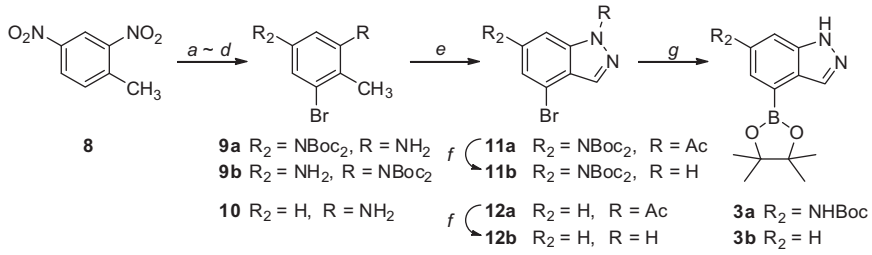
Figure 1. (a) Superposition of the ATP-binding sites of the 3D-aligned JAK isozymes bound to the same inhibitor molecule, CMP. Color code: atom type (JAK1), green (JAK2), pink (JAK3). Tyk2 structure, which showed significant structural difference compared with others, was omitted for the sake of clarity. (b) Molecular surface of JAK1 ATP-binding site. The putative inhibitor binding site is indicated by block arrows.



Scheme 1. Synthesis of the title compounds **1a**–**1f**. Reagents and conditions: (a) $ArB(OR)_2$, K_2CO_3, $Pd(PPh_3)_2$, toluene, 90 °C; (b) 2 N HCl, THF, rt.



Scheme 2. Preparation of the 3-alkynolyl-5-bromo-indazole-7-carboxamides **2a**–**2c**. Reagents and conditions: (a) NBS, DMF, rt; (b) KOAc, Ac_2O, $CHCl_3$, 0 °C to rt; iso-amyl nitrite, 18-crow-6; (c) 6 N HCl, MeOH, rt; (d) LiOH, H_2O/THF, 50 °C; (e) $(COCl)_2$, CH_2Cl_2; NH_3, MeOh; (f) Boc_2O, DMAP, CH_2Cl_2, rt; (g) NIS, DMF, rt; (h) propargyl alcohol or 3-butynyl alcohol, $Pd(PPh_3)_2$, CuI, TEA, CH_2Cl_2, rt.



Scheme 3. Preparation of the 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indazole derivatives **3a**–**3b**. Reagents and conditions: (a) NBS, DMF, H_2SO_4, 80 °C; (b) iron power, concd HCl, EtOH; (c) Boc_2O, BMAP, CH_2Cl_2, rt; (d) H_2, Pd/C, MeOH; (e) KOAc, Ac_2O, $CHCl_3$, 0 ° to rt; 18-crow-6, iso-amyl nitrite, rt to 100 °C; (f) 6 N HCl, MeOH, rt; (g) bis(pinacolato)diboron, $PdCl_2(dppf)_2$, KOAc, DMSO, 80 °C.

under the same reaction conditions described above to give a mixture of the cyclized products **11a** and **11b** in 81% yield. After deacetylation, **11a** was converted into **11b** in quantitative yield. Finally, 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indazole derivative **3a** was prepared by treatment of **11b** with bis(pinacoloato)diboron and $PdCl_2(dppf)_2$ in the presence of KOAc in 36% yield.[24] On the other hand, **3b** was prepared in an analogous manner starting from the commercially available 3-bromo-2-methylaniline **10** in 44% overall yield.

2.3. Biological activity of the title compounds

In vitro inhibitory activity of the indazole derivatives on the JAK isozymes was determined by Z′-LYTE™ Kinase Assay Kit-Tyr 6 Peptide (JAK1–JAK3) and Tyr 3 Peptide (Tyk2) (Invitrogen) according to the manufacturer's instruction, and%-inhibition of each JAK isozyme by 10 μM of the title compounds are summarized in Table 1.

As anticipated, it is clearly shown that the title compounds lack the inhibitory activity against JAK2, and their JAK1 selectivity over JAK2 (1.9 to >67.0) is much higher than that of the reported JAK1-selective inhibitors (6.4 fold).[12] Among the series, the unsubstituted 5-(4′-indazolyl)indazole-7-carboxamide derivatives (**1a** and **1b**) showed lowest inhibitory activity against JAK1 while the 4-alkynol-substituents significantly enhanced the JAK1-inhibitory activity of the corresponding derivatives (**1c**–**1f**). In particular, propargyl alcohol contributed more to the JAK1-inhibitory activity than butynol, which resulted in higher inhibitory activity of **1c** and **1d** compared with their but-3-yn-1-ol counterparts, **1e** and **1f**, respectively. The possible binding role of the 6′-NH_2 functionality is also noteworthy because those with 6′-NH_2 substituents (**1d** and **1f**) showed higher inhibitory activity compared with the unsubstituted derivatives (**1c** and **1e**). The title compounds exhibited similar level of inhibition against JAK3 with the exception of the propargyl alcohol-substituted derivatives **1c** and **1d**, which

showed almost two-fold decreased inhibitory activity against JAK3 compared with JAK1.

3. Conclusion

Taken together, the propargyl alcohol functionality endowed the 5-(4′-indazolyl)indazole-7-carboxamide scaffold with JAK1 selectivity over other JAK isozymes, particularly JAK2, and this observation supports our hypothesis that a polar group attached to a linear tether would serve as an isozyme-specific linear probe group, which would enable the resulting inhibitor to differentiate the ATP-binding sites of JAK1 from those of other isozymes. Further optimization study of the aklynol-substituted 5-(4′-indazolyl)indazole-7-carboxamide derivatives as JAK1-selective inhibitors are now under way and will be published elsewhere.

4. Experimental

4.1. Materials and general methods

Unless otherwise indicated, all chemicals and solvents were purchased from standard sources and used without further purification. Nuclear magnetic resonance spectra were recorded on a Bruker 400 AMX spectrometer (Karlsruhe, Germany) at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR with tetramethylsilane as the internal standard. Chemical shifts (δ) are given in parts per million (ppm) relative to the remaining protons of the deuterated solvents used as internal standard. Coupling constants *J* are reported in Hertz (Hz) and spin multiplicities are reported as s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), m (multiplet), or br s (broad singlet). TLC was performed on silica gel 60 F_{254} purchased from Merck. Column chromatography was performed using silica gel-60 (220–440 mesh) for flash chromatography. Mass spectrometric data (MS) were obtained by electronspray ionization (ESI). High resolution fast atom bombardment (FAB) mass spectra were recorded using a JEOL JMS-700 mass spectrometer at the Daegu center of KBSI, Korea. All tested compounds were ⩾95% purity, as determined by reverse phase HPLC. HPLC was performed on Agilent 1050 (Hewlett-Packard) equipment with variable wavelength (VW) UV detector using Polaris 5, C18-A 250 × 4.6 mm (Varian) column. Analytical conditions were as follows: gradient used was 20–25% acetonitrile in water containing 0.1% formic acid (0–8 min), 25–35% acetonitrile in water containing 0.1% formic acid (8–18 min), 35% acetonitrile in water containing 0.1% formic acid (18–25 min), 35–80% acetonitrile in water containing 0.1% formic acid (25–40 min), 80–100% acetonitrile in water containing 0.1% formic acid (40–45 min), 100% acetonitrile in water containing 0.1% formic acid (45–50 min), 100–20% acetonitrile in water containing 0.1% formic acid (50–54 min) and 20% acetonitrile in water containing 0.1% formic acid (54–60 min). Flow rate was 1 mL/min. UV was detected at 340 nm.

4.2. Syntheses of the title compounds

4.2.1. 1-(*N*,*N*-Di-Boc-amino)-5-bromo-4-methyl-3-nitrobenzene (9a)

To a solution of 1-methyl-2,4-dinitrobenzene (1.00 g, 5.50 mmol) in sulfuric acid (10 ml) was added NBS (1.17 g, 6.60 mmol) in three portions over 45 min while keeping the reaction temperature at 80 °C. Once the reaction was complete, the reaction mixture was cooled to rt. Ice-cold water and CH_2Cl_2 were added to the reaction mixture and, after phase separation, the organic layer was washed with saturated aqueous $Na_2S_2O_3$ solution. Concentration of the organic layer under reduced pressure provided 1-bromo-2-methyl-3,5-dinitrobenzene as a white solid (1.40 g, 98% yield): ^{1}H NMR (400 MHz, CD_3COCD_3) δ 8.72 (d, *J* = 2.3 Hz, 1H), 8.68 (d, *J* = 8.3 Hz, 1H), 2.66 (s, 3H).

1-Bromo-2-methyl-3,5-dinitrobenzene (1.00 g, 3.83 mmol) obtained above was dissolved in a mixture of EtOH (10 ml) and THF (2 ml), and iron powder was added at 0 °C. After stirring at 80 °C for 2 h, the reaction mixture was cooled to rt, diluted with a mixture of CH_2Cl_2 and water, and filtered through Celite washing with CH_2Cl_2. Organic phase of the filtrate was mixed with water and then treated with K_2CO_3 until the aqueous phase became basic. After phase separation, the organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give a yellow residue. Purification by column chromatography on silica gel provided an inseparable mixture of 3-bromo-4-methyl-5-nitroaniline and 3-bromo-2-methyl-5-nitroaniline, in 3:1 ratio (0.65 g, 74% combined yield).

The nitroaniline derivatives thus obtained were dissolved in CH_2Cl_2 (12 ml) and treated with DMAP (1.04 g, 8.49 mmol) and Boc_2O (1.85 g, 8.49 mmol). After stirring for 3 h at rt, the reaction mixture was concentrated under reduced pressure and the residue was purified by column chromatography on silica gel (hexanes/CH_2Cl_2/Et_2O = 100:100:1) to give an inseparable mixture of di-*tert*-butyl (5-bromo-4-methyl-3-nitrophenyl)carbamate and di-*tert*-butyl (3-bromo-2-methyl-5-nitrophenyl)carbamate as a yellow solid (0.99 g, 81% yield).

To the solution of the intermediate obtained above in MeOH (10 ml) was added Pd/C (0.05 g), and the reaction flask was charged with H_2 by using a balloon. The reaction mixture was stirred for 4 h at rt, and filtered through a Celite pad. The filtrate was concentrated under reduced pressure and the residue was purified by column chromatography on silica gel (hexanes/EtOAc = 5:1) to give the desired compound **9a** as a yellow powder (0.37 g, 40%

Table 1
%-Inhibition of the JAK isozymes by 10 μM of the title compounds

Compd	%-Inhibition at 10 μM[a]				Selectivity[b]	
	JAK1	JAK2	JAK3	Tyk2	JAK1/JAK2	JAK1/JAK3
1a	22.2 ± 1.2	1.2 ± 0.3	22.5 ± 2.3	7.8 ± 0.8	18.5	1.0
1b	17.4 ± 1.6	9.1 ± 1.6	15.7 ± 2.1	12.1 ± 1.2	1.9	1.1
1c	46.7 ± 2.4	2.2 ± 0.6	24.9 ± 1.8	2.5 ± 0.6	21.2	1.9
1d	67.0 ± 3.2	−0.4 ± 0.1	38.1 ± 2.7	0.6 ± 0.1	>67.0	1.8
1e	27.3 ± 2.5	6.7 ± 0.9	32.3 ± 3.4	9.3 ± 2.3	4.1	0.8
1f	59.8 ± 4.0	2.5 ± 1.2	48.1 ± 2.5	27.2 ± 2.7	23.9	1.2
Inh[c]	4.3	1.3	12.6	5.1	0.3	2.9
(IC_{50}, nM)	(3.3)[d]	(2.5)[d]	(10.9)[d]	(3.6)[d]		

[a] Each experiment was repeated at least three times.
[b] Selectivity = % inhibition of JAK1 at 10 μM/% inhibition of JAK2 (or JAK3) at 10 μM.
[c] Pan-JAK inhibitor, 'pyridine 6'. [25]
[d] IC_{50} values reported in Ref. 25.

yield): ^{1}H NMR (400 MHz, CD_3COCD_3) δ 6.70 (d, J = 2.0 Hz, 1H), 6.56 (d, J = 2.0 Hz, 1H), 4.93 (br s, 2H), 2.24 (s, 3H), 1.45 (s, 18H).

4.2.2. Di-*tert*-butyl (4-bromo-1*H*-indazol-6-yl)carbamate (11b)

To a solution of di-*tert*-butyl (3-amino-5-bromo-4-methylphenyl)carbamate (**9a**) (0.37 g, 0.91 mmol) in $CHCl_3$ (3 ml) was added KOAc (0.09 g, 0.96 mmol) at rt. After cooling to 0 °C, Ac_2O (0.17 ml, 1.82 mmol) was added and the reaction mixture was stirred at rt until white solid formed (ca. 15 min). Then, 18-crown-6 (0.05 g, 0.18 mmol) and isopentyl nitrite (0.27 ml, 2.00 mmol) were added, and the reaction mixture was stirred at 65 °C for 18 h. After cooling to rt, the reaction mixture was diluted with $CHCl_3$ and washed sequentially with saturated aqueous $NaHCO_3$ solution and brine. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (hexanes/EtOAc = 6:1) to give di-*tert*-butyl (1-acetyl-4-bromo-1*H*-indazol-6-yl)carbamate **11a** (0.19 g, 45% yield).

Compound **11a** obtained above (0.19 g, 0.41 mmol) was dissolved in MeOH (5 mL) and treated with 6 N HCl (2 ml). The reaction mixture was stirred at rt for 2 h and then concentrated under reduced pressure. The residue was taken with EtOAc and washed with water. The organic layer was concentrated to give the desired di-*tert*-butyl (4-bromo-1*H*-indazol-6-yl)carbamate (**11b**) as a bright brown solid (0.15 g, 89% yield): ^{1}H NMR (400 MHz, CD_3COCD_3) δ 8.33 (d, J = 0.5 Hz, 1H), 8.23 (s, 1H), 7.56 (d, J = 1.5 Hz, 1H), 1.41 (s, 18H); ^{13}C NMR (100 MHz, acetone-d_6) δ 171.7, 152.1, 142.2, 140.1, 139.3, 128.7, 125.8, 114.8, 113.7, 83.6, 28.0, 23.0.

4.2.3. 4-Bromo-1*H*-indazole (12b)

To a solution of 3-bromo-2-methylaniline (**10**) (0.30 g, 1.61 mmol) in $CHCl_3$ (5 ml) was added KOAc (0.17 g, 1.69 mmol) at rt. After cooling to 0 °C, Ac_2O (0.30 ml, 3.22 mmol) was added and the reaction mixture was stirred at rt until white solid formed (ca. 15 min). Then, 18-crown-6 (0.085 g, 0.32 mmol) and isopentyl nitrite (0.48 mL, 3.54 mmol) were added, and the reaction mixture was stirred at 65 °C for 18 h. After cooling to rt, the reaction mixture was diluted with $CHCl_3$ and washed sequentially with saturated aqueous $NaHCO_3$ solution and brine. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (hexanes/EtOAc = 5:1) to give 1-(4-bromo-1*H*-indazol-1-yl)ethanone **12a** (0.19 g, 49% yield) and 4-bromo-1*H*-indazole **12b** (0.13 g, 40% yield).

Compound **12a** (0.19 g, 0.79 mmol) was dissolved in MeOH (4 mL) and treated with 6 N HCl (2 ml). The reaction mixture was stirred at rt for 2 h and then concentrated under reduced pressure. The residue was taken with EtOAc and washed with water. The organic layer was concentrated to give the desired 4-bromo-1*H*-indazole (**12b**) as a bright brown solid (0.14 g, 90% yield): ^{1}H NMR (400 MHz, CD_3COCD_3) δ ^{1}H NMR (400 MHz, acetone-d_6) δ 12.86 (br s, 1H), 8.05 (s, 1H), 7.63 (d, J = 8.1 Hz, 1H), 7.34 (d, J = 7.3 Hz, 1H), 7.29 (t, J = 7.8 Hz, 1H).

4.2.4. *tert*-Butyl (4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indazol-6-yl)carbamate (3a)

A solution of di-*tert*-butyl (4-bromo-1*H*-indazol-6-yl)carbamate (**11b**) in Et_2O was treated with 1.7 M *t*-BuLi (0.72 ml, 1.23 mmol) at −78 °C in a dropwise manner and stirred for 1 h. Pinacol borane (0.18 ml, 1.23 mmol) was slowly added and the reaction mixture was stirred for 1 h at −78 °C before being allowed to warm to rt. More anhydrous Et_2O was added to facilitate stirring. After stirring for 24 h, the resulting sticky mixture was diluted with Et_2O and transferred in portions with stirring to a precooled solution of 2 N HCl. After stirring for 30 min, the acidic mixture was extracted with Et_2O and the combined extracts were washed with 2 N NaOH. The combined base extracts were washed with brine, dried over $MgSO_4$, concentrated under reduced pressure and purified by column chromatography on silica gel (hexanes/Et_2O = 4:3) to gain **3a** as a white solid (0.046 g, 31% yield): ^{1}H NMR (400 MHz, CD_3COCD_3) δ 12.33 (br s, 1H), 8.68 (br s, 1H), 7.98 (s, 1H), 7.90 (d, J = 0.9 Hz, 1H), 7.50 (d, J = 1.5 Hz, 1H), 2.84 (s, 12H), 1.51 (s, 9H).

4.2.5. 4-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indazole (3b)

A solution of 4-bromo-1*H*-indazole (**12b**) (0.20 g, 1.02 mmol) in Et_2O (6 mL) was cooled to −78 °C and then treated with 1.7 M *t*-BuLi (1.78 ml, 3.06 mmol) slowly. The resulting cream color mixture was stirred for 1 h at -78 °C. Pinacol borane (0.44 ml, 3.06 mmol) was added to this solution in a dropwise manner and then the mixture was stirred for 1 h at −78 °C. The reaction mixture was stirred for additional 2 h at rt, and the resulting sticky mixture was taken with EtOAc. The organic layer was washed sequentially with saturated aqueous ammonium chloride solution and brine. The combined organic layer was separated, dried over $MgSO_4$ and concentrated under reduced pressure. The crude mixture was purified by column chromatography on silica gel (hexanes/Et_2O = 4:3) to obtain **3b** (0.11 g, 44% yield) as white powder: ^{1}H NMR (400 MHz, CD_3COCD_3) δ 8.36 (s, 1H), 7.72 (d, J = 8.3 Hz, 1H), 7.60 (d, J = 6.8 Hz, 1H), 7.38 (dd, J = 6.8, 8.3 Hz, 1H), 1.40 (s, 12H).

4.2.6. Methyl 2-amino-5-bromo-3-methylbenzoate (5)

To a solution of methyl 2-amino-3-methylbenzoate (**4**) (1.00 g, 6.10 mmol) in DMF (10 ml) was added NBS (1.10 g, 6.10 mmol), and the reaction mixture was stirred at rt for 6 h. The reaction mixture was diluted with EtOAc (10 ml) and washed with saturated aqueous Na_2CO_3 solution (10 ml × 3). The organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (hexanes/EtOAc = 100:1) to give compound **5** as pale gray solid (0.93 g, 62% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.88 (d, J = 2.3 Hz, 1H), 7.28 (d, J = 2.3 Hz, 1H), 5.82 (s, 2H), 3.86 (s, 3H), 2.15 (s, 3H).

4.2.7. Methyl 5-bromo-1*H*-indazole-7-carboxylate (6b)

To a stirred solution of methyl 2-amino-5-bromo-3-methylbenzoate (**5**) (0.50 g, 2.20 mmol) in $CHCl_3$ (5 ml) was added KOAc (0.22 g, 2.30 mmol) at rt. After cooling to 0 °C, Ac_2O (0.40 ml, 4.20 mmol) was added and the reaction mixture was stirred at rt until white solid formed (ca. 15 min). Then, 18-crown-6 (0.12 g, 0.40 mmol) and isopentyl nitrite (0.60 ml, 4.80 mmol) were added, and the reaction mixture was stirred at 65 °C for 18 h. After cooling to rt, the reaction mixture was diluted with $CHCl_3$ and washed sequentially with saturated aqueous $NaHCO_3$ solution and brine. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (hexanes/EtOAc = 6:1) to give **6a** (0.30 g, 46% yield) and **6b** (0.20 g, 35% yield).

Compound **6a** (0.30 g, 1.00 mmol) was dissolved in MeOH (5 mL) and treated with 6 N HCl (2 ml). The reaction mixture was stirred at rt for 2 h and then concentrated under reduced pressure. The residue was taken with EtOAc and washed with water. The organic layer was concentrated to give the desired methyl 5-bromo-1*H*-indazole-7-carboxylate (**6b**) as a bright brown solid (0.25 g, 98% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 12.85 (s, 1H), 8.16 (d, J = 1.5 Hz, 1H), 8.13 (t, J = 1.5 Hz, 1H), 8.09 (d, J = 1.5 Hz, 1H), 4.04 (s, 3 H).

4.2.8. *tert*-Butyl (5-bromo-1*H*-indazole-7-carbonyl)carbamate (2a)

To a stirred solution of *tert*-butyl (5-bromo-1*H*-indazole-7-carbonyl)carbamate (**6b**) (0.55 g, 2.20 mmol) in a mixture of THF

(2 ml) and water (8 ml), LiOH (0.20 g, 8.60 mmol) was added, and the reaction mixture was stirred at 50 °C for 4 h. 2 N HCl was added to the reaction mixture to adjust pH to 2, and the resulting white solid was filtered. The solid was dissolved in CH_2Cl_2 (5 ml) and treated with $(COCl)_2$ (0.20 ml, 2.20 mmol). After stirring for 1 h, the reaction mixture was concentrated under reduced pressure. The residue was taken with saturated methanolic ammonia (5 ml), and stirred for 4 h at rt. The reaction mixture was concentrated under reduced pressure, and the residue was purified by column chromatography on silica gel (hexanes/acetone = 2:1) to give the 5-bromo-1*H*-indazole-7-carboxamide (0.45 g, 85% yield) as a pale brown solid: ^{1}H NMR (400 MHz, MeOD) δ (ppm) 11.24 (s, 1H), 10.63 (s, 2H), 8.16 (d, J = 1.5 Hz, 1H), 8.12 (t, J = 1.5 Hz, 1H), 8.09 (d, J = 1.5 Hz, 1H).

5-Bromo-1*H*-indazole-7-carboxamide (0.45 g, 1.90 mmol) obtained above was dissolved in CH_2Cl_2 and treated with Boc_2O (0.42 g, 1.90 mmol) and DMAP (0.23 g, 1.90 mmol). After stirring at rt for 6 h, the reaction mixture was concentrated under reduced pressure, and the residue was purified by column chromatography on silica gel (hexanes/EtOAc = 2:1) to give the desired *tert*-butyl (5-bromo-1*H*-indazole-7-carbonyl)carbamate (**2a**) as a pale brown oil (0.61 g, 95% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.18 (d, J = 1.5 Hz, 1H), 8.14 (t, J = 1.5 Hz, 1H), 8.10 (d, J = 1.5 Hz, 1H), 1.50 (s, 9H).

4.2.9. *tert*-Butyl (5-bromo-3-iodo-1*H*-indazole-7-carbonyl)carbamate (7)

To a stirred solution of *tert*-butyl (5-bromo-1*H*-indazole-7-carbonyl)carbamate (**2a**) (0.61 g. 1.80 mmol) in DMF (5 ml) was added NIS (0.24 g, 1.80 mmol). After stirring at rt for 6 h, the reaction mixture was diluted with EtOAc (10 ml) and washed with saturated aqueous Na_2CO_3 solution (10 ml × 3). The organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (hexanes/EtOAc = 2:1) to give *tert*-butyl (5-bromo-3-iodo-1*H*-indazole-7-carbonyl)carbamate (**7**) (0.45 g, 54% yield) as a pale gray solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 12.86 (s, 1H), 10.58 (s, 1H), 8.44 (d, J = 1.5 Hz, 1H), 8.30 (d, J = 1.5 Hz, 1H), 1.50 (s, 9H).

4.2.10. *tert*-Butyl (5-bromo-3-(3-hydroxyprop-1-yn-1-yl)-1*H*-indazole-7-carbonyl) carbamate (2b) and *tert*-butyl (5-bromo-3-(4-hydroxybut-1-yn-1-yl)-1*H*-indazole-7-carbonyl)carbamate (2c)

To a stirred solution of *tert*-butyl (5-bromo-3-iodo-1*H*-indazole-7-carbonyl)carbamate (**7**) (0.45 g, 1.00 mmol) in CH_2Cl_2 were added $Pd(PPh_3)_4$ (0.23 g, 0.20 mmol), CuI (0.095 g, 0.50 mmol), propargyl alcohol or 3-butynyl alcohol (1.00 mmol), and TEA (0.30 ml, 2.00 mmol). After stirring at rt for 24 h, the reaction mixture was diluted with CH_2Cl_2 and washed with brine (10 ml × 3). The organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (hexanes/acetone = 2:1) to give compound **2b** or **2c**.

Compound **2b** (0.14 g, 36% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 12.85 (s, 1H), 10.58 (s, 1H), 8.40 (d, J = 1.5 Hz, 1H), 8.28 (d, J = 1.5 Hz, 1H), 3.62–3.64 (m, 2H), 2.28–2.30 (m, 2H), 1.50 (s, 9H).

Compound **2c** (0.12 g, 30% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 12.85 (s, 1H), 10.58 (s, 1H), 8.40 (d, J = 1.5 Hz, 1H), 8.28 (d, J = 1.5 Hz, 1H), 4.12–4.16 (m, 2H), 1.49 (s, 9H).

4.2.11. 5-(4′-Indazolyl)indazole-7-carboxamides (1a–1f)

To a stirred mixture of the compound **2** (1.00 mmol) in toluene (10 ml) were added K_2CO_3 (1.50 mmol), the compound **3** (1.00 mmol), and $Pd(PPh_3)_4$ (0.20 mmol). After stirring at 90 °C for 12 h, the reaction mixture was cooled to rt and diluted with EtOAc (10 ml). The organic layer was washed with brine (10 ml × 3), dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was taken with THF (10 ml) and treated with 2 N HCl (5 ml). After stirring at rt for 4 h, the reaction mixture was neutralized with 2 N NaOH and extracted with EtOAc (10 ml × 3). The combined organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH = 20:1) to give the desired compound **1** in 37–68% yield.

Compound **1a** (0.19 g, 68% yield): ^{1}H NMR (400 MHz, MeOD) δ (ppm) 12.86 (br s, 2H), 8.14 (d, J = 1.5 Hz, 1H), 8.12 (t, J = 1.5 Hz, 1H), 8.08 (d, J = 1.5 Hz, 1H), 8.05 (s, 1H), 7.61 (d, J = 7.8 Hz, 1H), 7.52 (br s, 2H), 7.32 (d, J = 7.8 Hz, 1H), 7.28 (t, J = 7.8 Hz, 1H); LC/MS (ESI) m/z found: 278.2 $[M+H]^+$; calcd for $C_{15}H_{11}N_5O$: 278.10.

Compound **1b** (0.12 g, 41% yield): ^{1}H NMR (400 MHz, MeOD) δ (ppm) 12.86 (br s, 2H), 8.14 (d, J = 1.5 Hz, 1H), 8.12 (t, J = 1.5 Hz, 1H), 8.08 (d, J = 1.5 Hz, 1H), 8.05 (s, 1H), 7.90 (d, J = 1.5 Hz, 1H), 7.52 (br s, 2H), 7.85 (d, J = 1.5 Hz, 1H), 4.95 (br s, 2H); LC/MS (ESI) m/z found: 293.3 $[M+H]^+$; calcd for $C_{15}H_{12}N_6O$: 293.11.

Compound **1c** (0.13 g, 39% yield): ^{1}H NMR (400 MHz, MeOD) δ (ppm) 12.86 (br s, 2H), 8.38 (d, J = 1.5 Hz, 1H), 8.21 (d, J = 1.5 Hz, 1H), 8.04 (s, 1H), 7.60 (d, J = 7.8 Hz, 1H), 7.52 (br s, 2H), 7.32 (d, J = 7.8 Hz, 1H), 7.28 (t, J = 7.8 Hz), 4.95 (br s, 2H), 4.12–4.16 (m, 2H); LC/MS (ESI) m/z found: 332.2 $[M+H]^+$; calcd for $C_{18}H_{13}N_5O_2$: 332.11.

Compound **1d** (0.13 g, 37% yield): ^{1}H NMR (400 MHz, MeOD) δ (ppm) 12.86 (br s, 2H), 8.38 (d, J = 1.5 Hz, 1H), 8.21 (d, J = 1.5 Hz, 1H), 8.04 (s, 1H), 7.85 (d, J = 1.5 Hz, 1H), 7.79 (d, J = 1.5 Hz, 1H), 7.52 (br s, 2H), 4.95 (br s, 2H), 4.12–4.16 (m, 2H); LC/MS (ESI) m/z found: 347.3 $[M+H]^+$; calcd for $C_{18}H_{14}N_6O_2$: 347.12

Compound **1e** (0.12 g, 38% yield): ^{1}H NMR (400 MHz, MeOD) δ (ppm) 12.86 (br s, 2H), 8.38 (d, J = 1.5 Hz, 1H), 8.21 (d, J = 1.5 Hz, 1H), 8.04 (s, 1H), 7.59 (d, J = 7.8 Hz, 1H), 7.52 (br s, 2H), 7.31 (d, J = 7.8 Hz, 1H), 7.26 (t, J = 7.8 Hz), 4.95 (br s, 2H), 4.12–4.16 (m, 2H), 2.26–2.30 (m, 2H); LC/MS (ESI) m/z found: 346.5 $[M+H]^+$; Calcd. for $C_{19}H_{15}N_5O_2$: 346.12

Compound **1f** (0.13 g, 37% yield): ^{1}H NMR (400 MHz, MeOD) δ (ppm) 12.86 (br s, 2H), 8.38 (d, J = 1.5 Hz, 1H), 8.21 (d, J = 1.5 Hz, 1H), 8.04 (s, 1H), 7.85 (d, J = 1.5 Hz, 1H), 7.79 (d, J = 1.5 Hz, 1H), 7.52 (br s, 2H), 4.95 (br s, 2H), 4.12–4.16 (m, 2H), 2.26–2.29 (m, 2H); LC/MS (ESI) m/z found: 362.4 $[M+H]^+$; calcd for $C_{19}H_{16}N_6O_2$: 362.13.

Acknowledgments

This research was supported by a grant of the Korean Health Technology R&D project, Ministry of Health & Welfare, Republic of Korea (A111698), by Priority Research Centers Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2009-0093824), and by a grant from Next Generation Bio-Green 21 PJ007982 (Korea Rural Development Administration).

References and notes

1. (a) O'Shea, J. J. *Immunity* **1997**, *7*, 1; (b) Pesu, M.; Laurence, A.; Kishore, N.; Wzillich, S. H.; Chan, G.; O'Shea, J. J. *Immunol. Rev.* **2008**, *223*, 132.
2. O'Shea, J. J.; Pesu, M.; Borie, D. C.; Changelian, P. S. *Nat. Rev. Drug Disc.* **2004**, *3*, 555.
3. (a) Clark, S. C.; Kamen, R. *Science* **1987**, *236*, 1229; (b) Metcalf, D. *Nature* **1989**, *339*, 27.
4. Rochman, Y.; Spolski, R.; Leonard, W. J. *Nat. Rev. Immunol.* **2009**, *9*, 480.
5. (a) Wrobleski, S. T.; Pitts, W. J. Advances in the Discovery of Small Molecule JAK3 Inhibitors. In *Annual Reports in Medicinal Chemistry*; Macor, J. E., Ed.; Elsevier Inc.: San Diego, 2009; p 247. Vol. 44, Chapter 12; (b) Changelian, P. S.; Flanagan, M. E.; Ball, D. J.; Kent, C. R.; Magnuson, K. S.; Martin, W. H.; Rizzuti, B. J.; Sawyer, P. S.; Perry, B. D.; Brissette, W. H.; McCurdy, S. P.; Kudlacz, E. M.; Conklyn, M. J.; Elliott, E. A.; Koslov, E. R.; Fisher, M. B.; Strelevitz, T. J.; Yoon, K.; Whipple, D. A.; Sun, J.; Munchhof, M. J.; Doty, J. L.; Casavant, J. M.; Blumenkopf,

T. A.; Hines, M.; Brown, M. F.; Lillie, B. M.; Subramanyam, C.; Shang-Poa, C.; Milici, A. J.; Beckius, G. E.; Moyer, J. D.; Su, C.; Woodworth, T. G.; Gaweco, A. S.; Beals, C. R.; Littman, B. H.; Fisher, D. A.; Smith, J. F.; Zagouras, P.; Magna, H. A.; Saltarelli, M. J.; Johnson, K. S.; Nelms, L. F.; Des Etages, S. G.; Hayes, L. S.; Kawabata, T. T.; Finco-Kent, D.; Baker, D. L.; Larson, M.; Si, M. S.; Paniagua, R.; Higgins, J.; Holm, B.; Reitz, B.; Zhou, Y. J.; Morris, R. E.; O'Shea, J. J.; Borie, D. C. *Science* **2003**, *302*, 875.

6. van der Heijde, D.; Tanaka, Y.; Fleischmann, R.; Keystone, E.; Kremer, J.; Zerbini, C.; Cardiel, M. H.; Cohen, S.; Nash, P.; Song, Y. W.; Tegzová, D.; Wyman, B. T.; Gruben, D.; Benda, B.; Wallenstein, G.; Krishnaswami, S.; Zwillich, S. H.; Bradley, J. D.; Connell, C. A. *Arthritis Rheum.* **2013**, *65*, 559.
7. Rodig, S. J.; Meraz, M. A.; White, J. M.; Lampe, P. A.; Riley, J. K.; Arthur, C. D.; King, K. L.; Sheehan, K. C.; Yin, L.; Pennica, D.; Johnson, E. M., Jr.; Schreiber, R. D. *Cell* **1998**, *93*, 373.
8. Shimoda, K.; Kouji, K.; Aoki, K.; Matsuda, T.; Miyamoto, A.; Shibamori, M.; Yamashita, M.; Numata, A.; Takase, K.; Kobayashi, S.; Shibata, S.; Asano, Y.; Gondo, H.; Sekiguchi, K.; Nakayama, K.; Nakayama, T.; Okamura, T.; Okamura, S.; Niho, Y.; Nakayama, K. *Immunity* **2000**, *13*, 561.
9. Parganas, E.; Wang, D.; Stravopodis, D.; Topham, D. J.; Marine, J. C.; Teglund, S.; Vanin, E. F.; Bodner, S.; Colamonici, O. R.; van Deursen, J. M.; Grosveld, G.; Ihle, J. N. *Cell* **1998**, *93*, 385.
10. Smolen, J. S.; Beaulieu, A.; Rubbert-Roth, A.; Ramos-Remus, C.; Rovensky, J.; Alecock, E.; Woodworth, T.; Alten, R. *Lancet* **2008**, *371*, 987.
11. Haan, C.; Rolvering, C.; Raulf, F.; Kapp, M.; Druckes, P.; Thoma, G.; Behrmann, I.; Zerwes, H. G. *Chem. Biol.* **2011**, *18*, 314.
12. Kulagowski, J. J.; Blair, W.; Bull, R. J.; Chang, C.; Deshmukh, G.; Dyke, H. J.; Eigenbrot, C.; Ghilardi, N.; Gibbons, P.; Harrison, T. K.; Hewitt, P. R.; Liimatta, M.; Hurley, C. A.; Johnson, A.; Johnson, T.; Kenny, J. R.; Kohli, P. B.; Maxey, R. J.; Mendonca, R.; Mortara, K.; Murray, J.; Narukulla, R.; Shia, S.; Steffek, M.; Ubhayakar, S.; Ultsch, M.; van Abbema, A.; Ward, S. I.; Waszkowycz, B.; Zak, M. *J. Med. Chem.* **2012**, *55*, 5901.
13. Zak, M.; Mendonca, R.; Balazs, M.; Barrett, K.; Bergeron, P.; Blair, W. S.; Chang, C.; Deshmukh, G.; DeVoss, J.; Dragovich, P. S.; Eigenbrot, C.; Ghilardi, N.; Gibbons, P.; Gradl, S.; Hamman, C.; Hanan, E. J.; Harstad, E.; Hewitt, P. R.; Hurley, C. A.; Jin, T.; Johnson, A.; Johnson, T.; Kenny, J. R.; Koehler, M. F. T.; Kohli, P. B.; Kulagowski, J. J.; Labadie, S.; Liao, J.; Liimatta, M.; Lin, J.; Lupardus, P. J.; Maxey, R. J.; Murray, J. M.; Pulk, R.; Rodriguez, M.; Savage, S.; Shia, S.; Steffek, M.; Ubhayakar, S.; Ultsch, M.; van Abbema, A.; Ward, S. I.; Xiao, L.; Xiao, Y. *J. Med. Chem.* **2012**, *55*, 6176.
14. Vardiman, J. W.; Harris, N. L.; Brunning, R. D. *Blood* **2002**, *100*, 2292.
15. Williams, N. K.; Bamert, R. S.; Patel, O.; Wang, C.; Walden, P. M.; Wilks, A. F.; Fantino, E.; Rossjohn, J.; Lucet, I. S. *J. Mol. Biol.* **2009**, *387*, 219.
16. Lucet, I. S.; Fantino, E.; Styles, M.; Bamert, R.; Patel, O.; Broughton, S. E.; Walter, M.; Burns, C. J.; Treutlein, H.; Wilks, A. F.; Rossjohn, J. *Blood* **2006**, *107*, 176.
17. Chrencik, J. E.; Patny, A.; Leung, I. K.; Korniski, B.; Emmons, T. L.; Hall, T.; Weinberg, R. A.; Gormley, J. A.; Williams, J. M.; Day, J. E.; Hirsch, J. L.; Kiefer, J. R.; Leone, J. W.; Fischer, H. D.; Sommers, C. D.; Huang, H. C.; Jacobsen, E. J.; Tenbrink, R. E.; Tomasselli, A. G.; Benson, T. E. *J. Mol. Biol.* **2010**, *400*, 413.
18. Norio, M.; Suzuki, A. *Chem. Rev.* **1995**, *95*, 2457.
19. Ikegami, H.; Jachmann, M.; Nokura, Y.; Iwata, C. WO 2007043677, 2007.
20. Castanedo, G.; Chuckowree, I.; Folkes, A.; Sutherlin, D. P.; Wan, N. C. WO 2009146406, 2009.
21. Sonogashira, K. *Organomet. Chem.* **2002**, *653*, 46.
22. Shieh, P.; Hangauer, M. J.; Bertozzi, C. R. *J. Am. Chem. Soc.* **2012**, *134*, 17428.
23. Mee, S. P. H.; Lee, V.; Baldwin, J. E.; Cowley, A. *Tetrahedron* **2004**, *60*, 3695.
24. Folkes, A. J.; Ahmadi, K.; Alderton, W. K.; Alix, S.; Baker, S. J.; Box, G.; Chuckowree, I. S.; Clarke, P. A.; Depledge, P.; Eccles, S. A.; Friedman, L. S.; Hayes, A.; Hancox, T. C.; Kugendradas, A.; Lensun, L.; Moore, P.; Olivero, A. G.; Pang, J.; Patel, S.; Pergl-Wilson, G. H.; Raynaud, F. I.; Robson, A.; Saghir, N.; Salphati, L.; Sohal, S.; Ultsch, M. H.; Valenti, M.; Wallweber, H. J. A.; Wan, N. C.; Wiesmann, C.; Workman, P.; Zhyvoloup, A.; Zvelebil, M. J.; Shuttleworth, S. J. *J. Am. Chem. Soc.* **2008**, *51*, 5522.
25. Thompson, J. E.; Cubbon, R. M.; Cummings, R. T.; Wicker, L. S.; Frankshun, R.; Cunningham, B. R.; Cameron, P. M.; Meinke, P. T.; Liverton, N.; Weng, Y.; DeMartino, J. A. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1219.