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# The Discovery of 2,5-Isomers of Triazole-pyrrolopyrimidine as Selective Janus Kinase 2 (JAK2) Inhibitors vs. JAK1 and JAK3

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## ABSTRACT

Members of the Janus kinase (JAK) family are potential therapeutic targets. Abnormal signaling by mutant JAK2 is related to hematological malignancy, such as myeloproliferative neoplasms (MPNs), and tyrosine kinase inhibitor (TKI)-resistance in non-small cell lung cancer (NSCLC). We discovered a potent and highly selective inhibitor of JAK2 over JAK1 and -3 based on the structure of 4-(2,5-triazole)-pyrrolopyrimidine. Among all triazole compounds tested, 2,5-triazole regioisomers more effectively inhibited JAK2 kinase activity than isomers with substitutions of various alkyl groups at the $R_2$ position, except for methyl-substituted 1,5-triazole, which was more potent than the corresponding 1,4- and 2,5-triazoles. None of the synthesized 1,4-isomers inhibited all three JAK family members. Compounds with phenyl or tolyl group substituents at the $R_1$ position were completely inactive compared with the corresponding analogues with a methyl substituted at the $R_1$ position. As a result of this structure-activity relationship, **54**, which is substituted with a cyclopropylmethyl moiety, exhibited significant inhibitory activity and selectivity ($IC_{50}$ = 41.9 nM, fold selectivity JAK1/2 10.6 and JAK3/2 58.1). Compound **54** also exhibited an equivalent inhibition of wild type JAK2 and the V617F mutant. Moreover, **54** inhibited the proliferation of HEL 92.1.7 cells, which carry JAK2 V617F, and gefitinib-resistant HCC827 cells. Compound **54** also suppressed STAT3 phosphorylation at Y705.



## Keywords

JAK2, JAK2V617F, JAK2 Inhibitor, STAT (Signal transducer and activator of transcription), 4-(2,5-Triazole)-pyrrolopyrimidine

## 1. Introduction

Janus kinases (JAKs) are intracellular non-receptor tyrosine kinases that play important roles in signaling processes in mammalian cells such as cell growth, survival, development and differentiation by activating receptors *via* the binding of various type 1/2 cytokine and growth factor hormones related to signal transduction.[1] Upon binding to their cognate receptors, cytokines activate JAK family members, facilitating the phosphorylation and recruitment of signal transducer and activator of transcription (STAT).[2] Four isoforms of JAK have been identified, including JAK1, JAK2, JAK3 and tyrosine kinase 2, also called TYK2.[3] Each JAK kinase has crucial roles in the pathogenesis of immune-related diseases and implications in hematological cancer signaling in various cells.[4] Accordingly, the JAK family and JAK/STAT have been investigated for their potential as therapeutic targets in drug discovery. JAK1 and TYK2 have been associated with various immune responses and their suppression.[5] JAK1 mediates pro-inflammatory cytokine downstream signaling *via* type I (IFN-α/β) and type II (IFN-γ) interferons and members of the IL-2, -4, and -10 family.[6] Because JAK1 and JAK3 control the cytokine signaling process during the development and differentiation of T cells, JAK3 activation is typically observed in autoimmune diseases such as rheumatoid arthritis (RA).[7] In contrast to other JAK kinases, JAK2 is involved in a pathway that contributes to the growth and progression of hematopoietic cancer cells,[8] and abnormal signals by mutated JAK2 induce hematological malignancies such as myeloproliferative neoplasms (MPNs).[9]

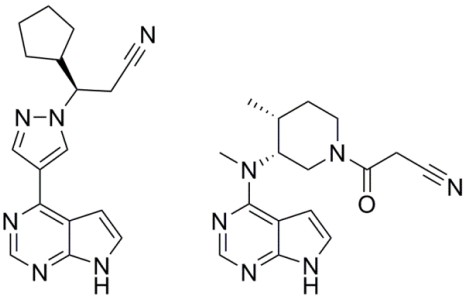
MPNs include polycythemia vera (PV), essential thrombocytosis (ET) and myelofibrosis (MF). The underlying pathogenic cause of MPNs is a valine to phenylalanine mutation at JAK2 amino acid 617 (JAK2V617F) in the JH2 domain; this mutation is present in nearly 95% of PV and more than 50% of ET and MF patients.[10] The JAK2V617F mutation is also observed with low allele frequency in lung cancer,[11] and based on this finding, Hedvat and coworkers evaluated the ability of JAK2 inhibitors to suppress lung cancer, demonstrating their potential in lung cancer combination therapy due to a synergistic effect.[12] In addition, the role of the JAK2V617F mutant in the pathogenesis of MPNs, including acute leukemia and other carcinomas,[13] has encouraged the development of JAK2 inhibitors

for targeted therapy of MPNs.[14] However, further studies are needed to elucidate the JAK2 pathway-related pathological events and to optimize therapeutic strategies. JAK family members JAK1, -2, and -3 exhibit significant structural similarity and a high level of sequence homology, and an understanding of their unique normal functions is a prerequisite for determining the JAK structure-function relationship and developing selectively targeted therapeutic agents.[15]

To resolve these structural and functional issues, the development of selective JAK2 inhibitors is crucial yet remains challenging. In this study, we designed and synthesized various analogues based on a 4-triazole-pyrrolopyrimidine skeleton for the discovery of selective and novel JAK2 inhibitors.

The pyrrolopyrimidine group of Ruxolitinib (**1**) and Tofacitinib (**2**), which are FDA approved as a JAK1/2 inhibitor and a JAK3 inhibitor, respectively, is a well-known pharmacophore.[16] Although Ruxolitinib (**1**) is a potent JAK1/2 inhibitor, it exhibits low selectivity between JAK1 and -2, and its chiral character complicates its synthesis (**Figure 1**). We therefore replaced the pyrazole with a triazole group to design target molecules without chirality. Gehringer *et al*. recently reported that the combinatorial chemistry of triazole-pyrrolopyrimidine analogues as pan-JAK inhibitors is limited to the 1,5-triazole isomer.[17] Triazole-pyrrolopyrimidine analogues were synthesized using TMS acetylene and a Sonogashira coupling reagent, and the TMS-protecting group was then removed to introduce the triazole structure. Using the same skeleton, we generated 3 isomers, 1,4-, 1,5- and 2,5-triazoles, by a substitution reaction at the triazole nitrogen and investigated their selectivity profiles. Methyl, phenyl and tolyl groups substituted at the triazole were generated using various alkyne intermediates with different chemical schemes, leading to the discovery of more potent JAK2 inhibitors with high selectivity over JAK1 and -3.

**Figure 1.**



Ruxolitinib (**1**)
JAK1 3.2 nM
JAK2 4.1 nM
JAK3 428 nM

Tofacitinib (**2**)
JAK1 3.2 nM
JAK2 4.1 nM
JAK3 1.6 nM

**Figure 1**. FDA-approved JAK inhibitors Ruxolitinib and Tofacitinib. The $IC_{50}$ values of Ruxolitinib (1) against JAK 1, -2 and -3 are 3.2, 4.1 and 428 nM, respectively, and those of Tofacitinib (2) are 3.2, 4.1 and 1.6 nM, respectively.

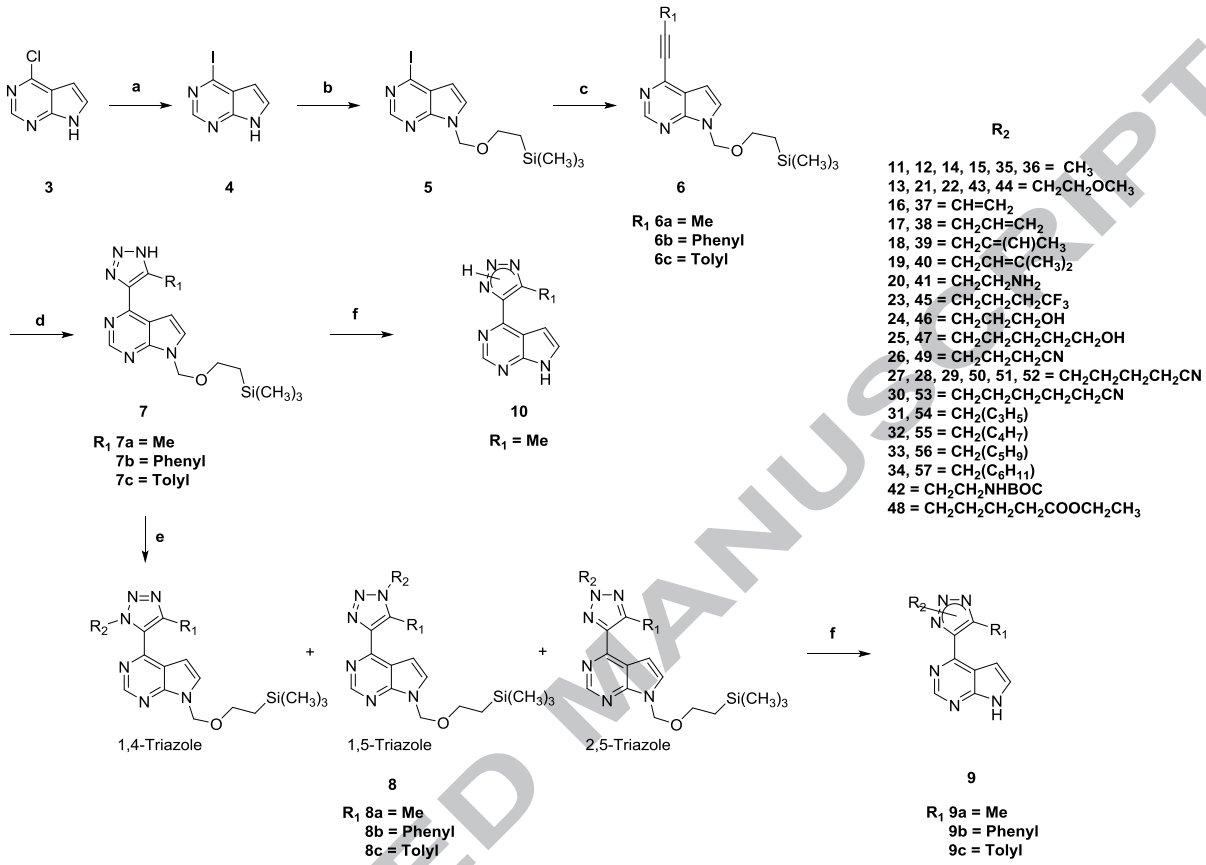
Here, we report the synthesis of novel 4-triazole-pyrrolopyrimidine derivatives, including identification of regioisomers with a JAK isoenzyme selectivity profile and their biological features, inhibition of the JAK2V617F mutant enzyme, and functional activity against HEL92.1.7 erythroblast cells (derived from MPN erythroleukemia) and non-small cell lung cancer (NSCLC) cells resistant to Gefitinib, an epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor.

## 2. Results and discussion

### *2.1. Chemistry*

For the synthesis of JAK2 inhibitors, the starting material 4-chloro-7H-pyrrolopyrimidine (**3**) was first transformed to the corresponding iodide compound **4** using hydriodic acid; the pyrrole-NH moiety was then protected by [2-(trimethylsilyl)ethoxy]methyl (SEM) to afford compound **5**. The SEM-protected pyrrolopyrimidine (**5**) was subjected to Sonogashira coupling reactions in the presence of TEA, CuI(I) and $Pd(PPh_3)_4$ and various alkyne moieties, such as propyne, phenylacetylene and 4-ethynyltoluene, to yield precursor compounds (**6a-c**) for the introduction of triazole groups at the pyrrolopyrimidine 4-position. 5-Membered cyclic triazoles (**7a-c**) were then generated by click chemistry with alkyne structures. Finally, different triazole regioisomers (**8**) were obtained through substitution reactions of compound **7** with various alkyl halides, $K_2CO_3$ and TBAB; subsequent deprotection reactions of the SEM group of intermediate compounds **7** and **8** yielded compounds **10** and **9**, respectively. Only 1,5- and 2,5-triazole isomers were obtained as the major and minor products, respectively, in alkyl substitution reactions in which $R_1$ was a methyl group (**7a**), except for methylation at the $R_2$ position, which generated all three isomers. However, 1,4-triazole isomers (**11**, **12** and **13**) were also obtained as minor products in alkylation reactions of the triazole moiety in which the $R_1$ position was substituted with phenyl and tolyl groups (**7b** and **7c**) as shown in **Scheme 1**. Among the 3 different regioisomers, only the 2,5-triazole isomers, the major products, inhibited the JAK2 enzyme with remarkable selectivity.

**Scheme 1**




Reagents and conditions : (a) i) HI, $H_2O$, rt, 48 h. ii) $NH_3$, $H_2O$, pH = 8; (b) 2-(Chloromethoxy)ethyltrimethylsilane, $K_2CO_3$, DMF, rt, 6 h; (c) $R_1$-alkyne, TEA, CuI(I), $Pd(PPh_3)_4$, DMF, rt, o/n; (d) $NaN_3$, DMF, 95 $^oC$, 5-7 h; (e) $R_2Br$, $K_2CO_3$, KOH, TBAB, ACN, rt, 3-24 h; (f) TBAF, THF, 60 $^oC$, 24-48 h

**Scheme 1.** The synthesis scheme of each triazole regioisomer substituted with various alkyl groups

## *2.2. Structural determination by NMR, including $^1$H, NOESY and XRD*

In the alkyl substitution reaction, the substituted triazole produced 2 major regioisomers: 1,5- and 2,5-triazole compounds. The structures of the triazole compounds were determined by XRD of compounds **21** and **43** (Supporting Information). $^1$H-NMR analysis of the 2 isomers revealed a striking difference in the chemical shifts of the 5$^{th}$ H ('b' in **Figure 2**) and NH proton of the pyrrolopyrimidine group between the 2 isomers, at 0.264 (7.477 ppm for 1,5-triazole and 7.213 ppm for 2,5-triazole) and 0.556 ppm (10.445 ppm for 1,5-triazole and 11.101 ppm for 2,5-triazole), respectively. In NOESY analysis, irradiation of the 'b' proton in each triazole isomer structure

produced significantly different patterns of effects on the other protons. The 'c', 'd', 'e', 'f' and 'g' protons of **43** (2,5-triazole) were enhanced, whereas only the 'c' and NH proton were affected in **21** (1,5-triazole). These data suggest that the methyl and methoxyethyl groups of the 2,5-triazole isomer (**43**) are closer to the 'b' proton than are the corresponding groups of the 1,5-triazole isomer.

To elucidate the possible reasons for the differences in chemical shifts in the 'b' proton in the 1,5-triazole (**21**) and 2,5-triazole (**43**) derivatives, the electron density of each isomer was determined by calculating the electrostatic potential utilizing density functional theory (DFT), a general computational calculating method based on molecular properties without symmetry constraints, in Gaussian 09 Rev D.01.[18] In the Mulliken population analysis, we did not observe any remarkable differences in the electrostatic potential between these triazole isomers. However, when a more detailed hybrid calculation protocol in the form of the B3LYP (Beck3 Lee-Yang-Parr exchange functions) method[19] was applied with 6-31+G(d)* standard basis sets for expansion of the Kohn-Sham orbitals, different features of the electron density of the two triazole isomers were observed in the electrostatic potential maps, as shown in **Figure 2**. The electrostatic potential of the 'b' proton of 2,5-triazole (**43**) was calculated as 0.117167, a value higher than that of 0.094112 for 1,5-triazole (**21**). The differences in the patterns of partial electron density between 1,5- and 2,5-triazole may be responsible for the observed chemical shifts of the 'b' protons between the isomers. Thus, the 'b' proton of 1,5-triazole (**21**), which has a lower partial electron density, was shifted downfield. We also examined the electron density of the molecular orbitals by comparing HOMO and LUMO to compute these energy gaps between the 1,5- and 2,5-triazole isomers, which were calculated as 4.588 and 4.568 eV, respectively. As a soft molecule with 4.568 eV of small HOMO-LUMO energy gap value will be more polarizable than a hard molecule with 4.588 eV of large HOMO-LUMO energy gap value, as shown in Supporting Information, the calculated electrostatic potential sufficiently explains their polarizability and diffusive difference. The results of the electron density analysis were supported by the structural determination of the regioisomers of the remaining derivatives due to the chemical shift of the 'b' and NH protons in the $^{1}$H-NMR spectra. Moreover, we confirmed that the $^{1}$H-NMR data of the 1,5-triazole analogs in this study were consistent with the 1,5-triazole derivatives

reported by Gehringer et. al[17] as pan-JAK inhibitors.

**Figure 2.**

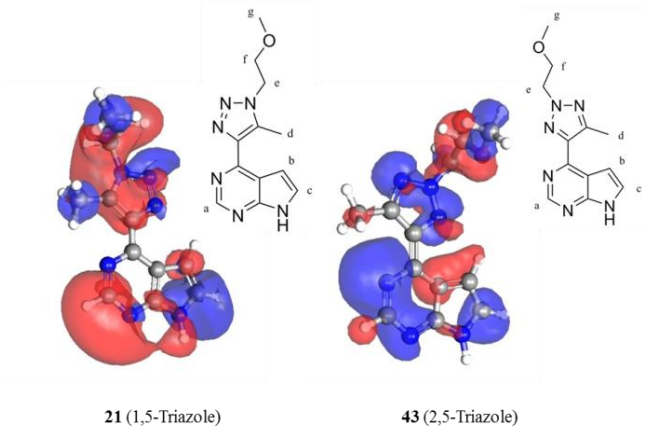



**Figure 2. Electrostatic potential maps of optimized structures of 1,5- (21) and 2,5-triazole (43) isomers.** Note that blue color denotes positive charge and red color negative charge.

### *2.3. In vitro enzymatic inhibitory activities*

Initially, all compounds were evaluated for their inhibitory activities against JAK kinase family members JAK1, -2 and -3 by screening at concentrations of 10 and 1 μM. **Table 1 (35-57)** and **Table S1 (10-34, see supporting information)** present the percent inhibition of each JAK family member at a 1 μM concentration; the $IC_{50}$ values of selected compounds are denoted in parentheses.

In general, 1) All synthesized 1,4-isomers (**11-13)** were inactive against all three JAK family members. 2) Most of the 1,5-triazole compounds (**14-34**) had weak or no inhibitory activity. 3) The 2,5-triazole regioisomers more effectively inhibited JAK2 kinase activity compared to the isomers with substitutions of various alkyl groups at the $R_2$ position, with the exception of the methyl-substituted 1,5-triazole **14**, which was more potent than the corresponding 1,4- and 2,5-triazoles (**11** and **36**, respectively). 4) In contrast to the corresponding methyl-substituted analogues, compounds with a phenyl or tolyl group at the $R_1$ position (**12**, **13**, **15**, **22**, **28**, **29**, **36**, **44, 51** and **52**) were

completely inactive.

Although no appreciable activities or SAR were detected in the series of 1,4- and 1,5-triazoles, potent and selective JAK2 inhibitory activities with clear SAR of the 2,5-triazole compounds was observed. Because most current JAK inhibitors contain a nitrile moiety, we attempted to introduce various substituents at the $R_2$ position, including electron-rich moieties such as alkene, amine, trifluoromethyl, hydroxyl and ester groups as well as a nitrile group. In the series of alkene-substituted derivatives (**37**-**40**), we initially observed a dramatic increase in JAK1 and -2 kinase inhibitory activity upon vinyl substitution (**37**), as opposed to methyl substitution (**35**), at the $R_2$ position. The pattern of inhibitory activities of the series of analogues was as follows: vinyl (**37**) < 3-methylbut-2-enyl (**40**) < allyl (**38**) < 2-methylallyl (**39**). In particular, **39**, which has an additional methyl group at the 2-position of the allyl group compared to **38**, exhibited a dramatic increase in JAK1 and -2 inhibitory activity, with $IC_{50}$ values of 142 and 67.4 nM (i.e., 7.6-fold increase for JAK2), respectively, and high selectivity over JAK3.

Based on the beneficial effect of alkene groups, **41**-**53** with various electron-rich groups at the termini of the $R_2$ substituents were synthesized and evaluated. Comparison of **38** and **41** or **42**, with similar lengths of $R_2$ substitution, revealed that an amine or NHBOC group decreased JAK inhibitory activity compared with an allyl substituent (**38**). Among other substituted alkyl moieties of similar length and with various electron-rich groups, compounds with methoxy (**43**), trifluorobutyl (**45**) and propanol (**46**) moieties exhibited only modest inhibitory activity against JAK2, with $IC_{50}$ values of 210, 96.3 and 156 nM, respectively. A similar inhibitory potency was observed in the case of the 3-trifluoromethyl-*n*-propyl-substituted compound **45**, ($IC_{50}$ = 82 nM at JAK1). Compound **47**, which shares a hydroxyl moiety with **46** but has a carbon chain elongated by two carbons, exhibited decreased inhibition against all JAK kinases.

Nitrile-substituted compounds **49**, **50** and **53**, which have different carbon chain lengths (4, 5 and 6, respectively), exhibited enhanced inhibition of JAK family members compared with other electron-rich substituted derivatives. In addition, inhibitory activity against JAK1 decreased with increasing

carbon chain length: 4 (**49**) > 5 (**50**) > 6 (**53**). However, inhibitory activity against JAK2 was optimum at a carbon chain length of 4 (**49**) or 5 (**50**), and compound **53**, with a 6 carbon chain length, displayed a 2.4-fold reduction in activity. Notably, compound **49** was the only inhibitor of JAK3 in this study with greater than 50% inhibition at 1 μM ($IC_{50}$ = 1.76 μM). Among the series of nitrile derivatives, compound **50** exhibited high JAK2 selectivity over JAK3 and 2.2-fold selectivity over JAK1.

Finally, various-sized cycloalkane moieties (compounds **54**-**57**) were evaluated because the representative JAK2 inhibitor Ruxolitinib (**1**) has a cyclopentane group as the key pharmacophore. Although the JAK1 inhibitory activities of compounds **54**-**57** were all similar, with 61-77% inhibition at 1 μM, JAK2 inhibition increased as the size of the cycloalkane ring decreased. Therefore, cyclopropylmethyl-substituted compound **54** was the most potent and selective inhibitor of JAK2, with an $IC_{50}$ value of 41.9 nM and 10.6- and 58.1-fold selectivity over JAK1 and JAK3, respectively.

Because the JAK2V617F mutant has been reported to be the etiological factor in JAK2-related MPN pathogenesis,[14] the ability of 7 compounds with potent JAK2 inhibitory activity to inhibit the mutant enzyme was evaluated (**Table S32, Supporting Information**). In general, the compounds exhibited lower inhibitory activity against the JAK2V617F mutant compared to wild type JAK2; the most potent inhibitors were pentanenitrile analogue **50** and cyclopropylmethyl analogue **54**, which exhibited 53.5% and 56.8% inhibition of JAK2V617F at a concentration of 100 nM. Based on these inhibitory activities against JAK2 and JAK2V617F, compound **50** and **54** were considered highly selective and novel JAK2 inhibitors.

Binding mode of Ruxolitinib (**1**) and compound **54** in the ATP binding site of JAK2 structure was described and CDOCKER interaction energy was calculated in **Figure S7, Supporting Information**.

**Table 1. Biological Inhibitory Activity against JAK Family Members, 2,5-triazole.**

| # | Regioisomer | $R_1$ | $R_2$ | JAK1[a] ($IC_{50}$[b]) | JAK2[a] ($IC_{50}$[b]) | JAK3[a] ($IC_{50}$[b]) |
|---|---|---|---|---|---|---|
| **35** | 2,5-Triazole | Me | $CH_3$ | 11.2 | 5.6 | 1.6 |
| **36** | 2,5-Triazole | 4-MePh | $CH_3$ | 9.1 | -0.6 | 4.1 |
| **37** | 2,5-Triazole | Me | $CH{=}CH_2$ | 45.4 | 60.4 | -8.7 |
| **38** | 2,5-Triazole | Me | $CH_2CH{=}CH_2$ | 50.7 | 82.3 (511) | 1.3 |
| **39** | 2,5-Triazole | Me | $CH_2C{=}(CH_2)CH_3$ | 81.1 (142) | 95.3 (67.4) | 15.6 |
| **40** | 2,5-Triazole | Me | $CH_2CH{=}C(CH_3)_2$ | 53.6 | 77.9 (786) | -4.3 |
| **41** | 2,5-Triazole | Me | $CH_2CH_2NH_2$ | 19.1 | 13.9 | 4.8 |
| **42** | 2,5-Triazole | Me | $CH_2CH_2NHBOC$ | 5.4 | 3.2 | -3.7 |
| **43** | 2,5-Triazole | Me | $CH_2CH_2OCH_3$ | 51.7 | 88.0 (310) | 8.3 |
| **44** | 2,5-Triazole | 4-MePh | $CH_2CH_2OCH_3$ | -3.8 | 2.5 | 7.1 |
| **45** | 2,5- | Me | $CH_2CH_2CH_2CF_3$ | 84.7 | 88.1 | 10.8 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Triazole | | | (82) | (96.3) | |
| **46** | 2,5-Triazole | Me | $CH_2CH_2CH_2OH$ | 62.6 | 86.7 (156) | 20.2 |
| **47** | 2,5-Triazole | Me | $CH_2CH_2CH_2CH_2CH_2OH$ | 53.6 | 60.4 | 2.0 |
| **48** | 2,5-Triazole | Me | $CH_2CH_2CH_2CH_2COOCH_2CH_3$ | 20.2 | 25.4 | -9.0 |
| **49** | 2,5-Triazole | Me | $CH_2CH_2CH_2CN$ | 89.0 (72) | 96.3 (73.5) | 51.1 (1,760) |
| **50** | 2,5-Triazole | Me | $CH_2CH_2CH_2CH_2CN$ | 78.4 (138) | 96.3 (63.6) | 14.7 |
| **51** | 2,5-Triazole | Ph | $CH_2CH_2CH_2CH_2CN$ | 7.7 | 11.1 | -1.1 |
| **52** | 2,5-Triazole | 4-MePh | $CH_2CH_2CH_2CH_2CN$ | 12.0 | 13.1 | 1.2 |
| **53** | 2,5-Triazole | Me | $CH_2CH_2CH_2CH_2CH_2CN$ | 81.6 (186) | 90.1 (165) | 6.4 |
| **54** | 2,5-Triazole | Me | $CH_2(C_3H_5)$ | 68.3 (444) | 96.1 (41.9) | 21.6 (2,440) |
| **55** | 2,5-Triazole | Me | $CH_2(C_4H_7)$ | 68.9 | 96.1 (62.7) | 22.5 |
| **56** | 2,5-Triazole | Me | $CH_2(C_5H_9)$ | 76.8 | 87.9 (71.8) | 11.1 |
| **57** | 2,5-Triazole | Me | $CH_2(C_6H_{11})$ | 60.6 | 32.0 | 6.7 |

[a] Arithmetic representation with 3 significant figures and determined by at least 3 separate tests. The percent inhibition of each JAK family member at a 1 μM concentration of the compound and the $K_m$ concentration of ATP is shown.

[b] Representative analogue of a potent inhibitor with the $IC_{50}$ value denoted in nanomolar units in parentheses.

[c] Negative values signify non-efficacy of inhibition at a 1 μM concentration

### *2.4. Cell growth inhibition*

Prior to further functional studies in cell models of JAK2-related MPN and gefitinib-resistant NSCLCs, the cell permeability of compounds **50** and **54** was assessed by PAMPA (Parallel Artificial Membrane Permeability Assay, High value > -4.07; Medium value -4.07 ~ -4.87; Low value < -4.87).[20] The results indicated moderate and high permeability of compounds **50** and **54**, with values of -4.38 ± 0.29 and -4.05 ± 0.41, respectively. In HEL92.1.7 cells, which are derived from an MPN erythroleukemia and carry the JAK2V617F mutation, the 7 representative analogues (except **45**) exhibited appreciable anti-proliferative efficacies of approximately 40-65% at a concentration of 10 μM **(Supporting Information)**; this was similar to Ruxolitinib (**1**), which has an $IC_{50}$ of 14.7 ± 0.25 μM in HEL92.1.7 cells. Although this result indicates a discrepancy between the inhibition of enzyme activity and cell proliferation, Ruxolitinib (**1**), which has a single-digit nanomolar $IC_{50}$ against JAK2, also only exhibited 55% inhibition at the same concentration in HEL 92.1.7 cells. Consistent with this gap in activity profiles between the enzyme and cells, an $EC_{50}$ value of 186 nM in HEL92.1.7 cells has been reported for Ruxolitinib (**1**).[21]

### *2.5. Western blotting*

Further functional study of compound **54** was performed in HCC827 WT and HCC827 GR cells (**Figure 3** and **Figure 4**). These cell lines are derived from wild type and gefitinib-resistant NSCLC, respectively, and the latter is a well-known form of EGFR inhibitor-resistant NSCLC. These cell types have been reported to be effectively suppressed by JAK2 inhibitors in combination with anticancer drugs such as TKIs or cisplatin.[22] When these cell lines were incubated with various concentrations of Ruxolitinib (**1**), a potent JAK1/2 inhibitor, or **54**, **1** exerted greater inhibition of proliferation in HCC827 GR cells (57.1% at 30 μM) compared to HCC827 WT cells (16.6% at 30 μM). Compound **54** inhibited the proliferation of HCC827 GR cells at 30 μM but exhibited no anti-proliferative effects against HCC827 WT cells. In addition, phosphorylation (Y705) of STAT3, which stimulates

homodimerization through mutual phosphotyrosine interactions in the SH2 domain and is a downstream signaling event of JAK2 activation,[23] was also inhibited by compound **54** at 30 μM in HCC827 GR cells. Interestingly, inhibition of STAT3 phosphorylation was not observed in HCC827 WT cells, whereas Ruxolitinib (**1**) significantly inhibited this signaling in both cell types.

**Figure 3.**

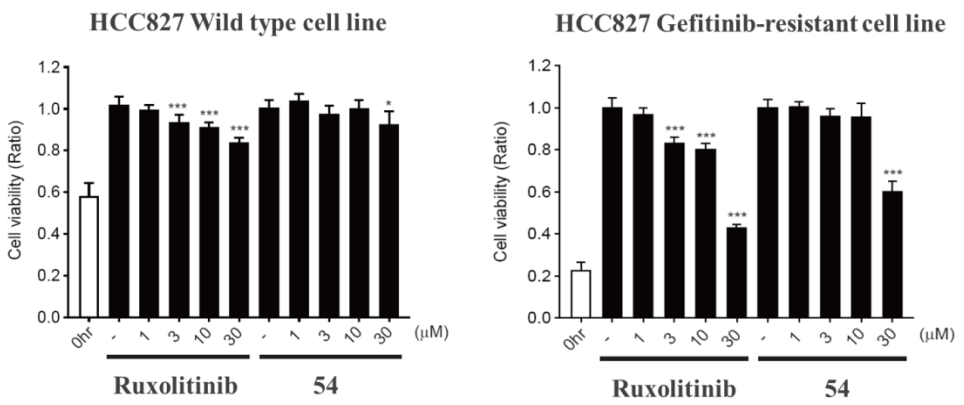



**Figure 3. Anti-proliferative effects in HCC827 and HCC827 GR cells**

**Figure 4.**

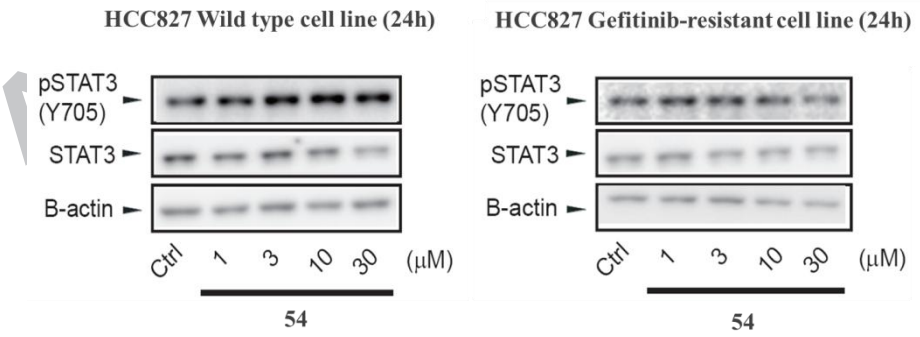



**Figure 4. Inhibition of STAT3 phosphorylation (Y705) in HCC827 and HCC827 GR cells**

## 3. Conclusion

Our results of synthetic and SAR studies of derivatives based on the 4-(2,5-triazole)-pyrrolopyrimidine structure have revealed potent and highly selective JAK2 inhibitors. According to SAR analysis and optimization of various alkyl-substituted groups with electron-rich moieties at the $R_2$ position, cyclopropylmethyl-substituted compound **54** ($IC_{50}$ = 41.9 nM and fold selectivity: JAK1/2 10.6 and JAK3/2 58.1) was found to be a novel potent and selective JAK2 inhibitor. Compound **54** also exhibits similar inhibitory potency against the JAK2V617F mutant. Moreover, **54** inhibits the proliferation of HEL 92.1.7 cells, which are derived from an MPN hematopoietic cancer carrying a JAK2 mutation, and Gefitinib-resistant NSCLC cells. Compound **54** also suppresses STAT3 phosphorylation at Y705 in the SH2 domain, a reciprocal interaction site of STAT3. The novel JAK2 selective inhibitors discovered in this study may be potential lead compounds for new drug discovery *via* further development of more potent and selective JAK2 inhibitors.

## 4. Experimental section

### *4.1. General Synthesis*

All reagents and solvents were purchased from commercial suppliers and used as supplied without further purification. Thin layer chromatography (TLC) was performed using fluorescent silica gel plates (60 $F_{254}$ from Merck) and visualized with short-wave UV light. Chromatographic purification was achieved using Kieselgel 60 (Merck) 0.040-0.063 mm column chromatography. Proton nuclear magnetic resonance spectroscopy was performed using a JEOL JNM-LA 300WB spectrometer at 300 MHz or JEOL JNM-ECX 400P spectrometer at 400 MHz, and spectra were collected in $CDCl_3$ or DMSO-$d_6$. Unless otherwise noted, chemical shifts are expressed as ppm downfield from internal tetramethylsilane (TMS) or relative ppm from DMSO (2.5 ppm). Data are reported as follows: chemical shift, integration, multiplicity (s, singlet; d, doublet; t, triplet; m, multiplet; br, broad), and coupling constants. Mass spectroscopy was carried out using MALDI-TOF and electrospray ionization (ESI) instruments.

**A. Procedure for 4-iodo-7H-pyrrolo[2,3-d]pyrimidine (4)**

4-Chloro-7H-pyrrolo[2,3-d]pyrimidine was dissolved in 57 wt. % hydriodic acid in $H_2O$. The solution was stirred for 48 h at room temperature, and the solid was removed by filtering. The suspension in cold water was brought to pH 8 with $NH_3$ (*aq*) solution. The solid was filtered, washed with water and dried.

**B. Procedure for 4-iodo-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidine (5)**

$K_2CO_3$ was added to a solution of 4-iodo-7H-pyrrolo[2,3-d]pyrimidine (**4**) in dry DMF and then stirred for 30 min at room temperature. After 30 min, 2-(trimethylsilyl)ethoxymethyl chloride was added drop-wise to the solution, and the reaction was stirred for 6 h at room temperature. For quenching, saturated $NH_4Cl$ aqueous solution was added. The mixture was poured into ethyl acetate and extracted twice. The combined organic layers were dried with $Na_2SO_4$, filtered and concentrated.

The residue was purified by silica gel column chromatography (hexane/ethyl acetate) to yield the desired compound.

**C-a. Procedure for 4-(prop-1-yn-1-yl)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidine (6a)**

$Pd(PPh_3)_4$, CuI(I) and $Et_3N$ were added to a solution of 4-iodo-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidine (**5**) in dried DMF. The mixture was bubbled with $N_2$ (g) for 15 min at room temperature. Propyne was bubbled into the solution for 1 h at room temperature, and the reaction was stirred overnight at room temperature. Finally, the solvent was removed *in vacuo*, and the residue was extracted twice with ethyl acetate and then washed with water. The combined organic layers were dried with $Na_2SO_4$, filtered and concentrated. The residue was purified by silica gel column chromatography (hexane/ethyl acetate) to yield the desired compound.

**C-b. Procedure for 4-(phenylethynyl)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidine (6b)**

$Pd(PPh_3)_4$, CuI(I) and $Et_3N$ were added to a solution of 4-iodo-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidine (**5**) in dried DMF. The mixture was bubbled with $N_2$ (g) for 15 min at room temperature. Phenylacetylene was bubbled into the solution for 1 h at room temperature, and the reaction was stirred overnight at room temperature. Finally, the solvent was removed *in vacuo*, and the residue was extracted twice with ethyl acetate and then washed with water. The combined organic layers were dried with $Na_2SO_4$, filtered and concentrated. The residue was purified by silica gel column chromatography (hexane/ethyl acetate) to yield the desired compound.

**C-c. Procedure for 4-(p-tolylethynyl)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidine (6c)**

$Pd(PPh_3)_4$, CuI(I) and $Et_3N$ were added to a solution of 4-iodo-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidine (**5**) in dried DMF. The mixture was bubbled with $N_2$ (g) for 15 min at room temperature. 4-Ethynyltoluene was bubbled into the solution for 1 h at room temperature, and

the reaction was stirred overnight at room temperature. Finally, the solvent was removed *in vacuo*, and the residue was extracted twice with ethyl acetate and then washed with water. The combined organic layers were dried with $Na_2SO_4$, filtered and concentrated. The residue was purified by silica gel column chromatography (hexane/ethyl acetate) to yield the desired compound.

**D-a. Procedure for 4-(5-methyl-1H-1,2,3-triazol-4-yl)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidine (7a)**

4-(Prop-1-yn-1-yl)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidine (**6a**) was dissolved in dried DMF, and $NaN_3$ was added. The solution was stirred for 5 h at 95 °C. After stirring, the solution was cooled to room temperature, and the solvent was removed *in vacuo*. The residue was added to 1 N HCl (*aq*) solution and extracted twice with ethyl acetate. The combined organic layers were dried with $Na_2SO_4$, filtered and concentrated. The residue was purified by silica gel column chromatography (hexane/ethyl acetate) to yield the desired compound.

**D-b. Procedure for 4-(5-phenyl-1H-1,2,3-triazol-4-yl)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidine (7b)**

4-(Phenylethynyl)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidine (**6b**) was dissolved in dried DMF, and $NaN_3$ was added. The solution was stirred for 7 h at 95 °C. After stirring, the solution was cooled to room temperature, and the solvent was removed *in vacuo*. The residue was added to 1 N HCl (*aq*) solution and extracted twice with ethyl acetate. The combined organic layers were dried with $Na_2SO_4$, filtered and concentrated. The residue was purified by silica gel column chromatography (hexane/ethyl acetate) to yield the desired compound.

**D-c. Procedure for 4-(5-tolyl-1H-1,2,3-triazol-4-yl)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidine (7c)**

4-(*p*-Tolylethynyl)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidine (**6c**) was dissolved in dried DMF, and $NaN_3$ was added. The solution was stirred for 7 h at 95 °C. After stirring, the solution was cooled to room temperature, and the solvent was removed *in vacuo*. The residue was

added to 1 N HCl (*aq*) solution and extracted twice with ethyl acetate. The combined organic layers were dried with $Na_2SO_4$, filtered and concentrated. The residue was purified by silica gel column chromatography (hexane/ethyl acetate) to yield the desired compound.

**E. General procedure for alkylation of triazoles (8a-c)**

$K_2CO_3$, KOH and TBAB were added to a solution of each triazole compound (**7a-c**) in ACN. Each solution was stirred for 30 min at room temperature; alkyl bromide was added, and the reaction proceeded for 3 – 24 h at room temperature. The solid was filtered and then washed with diethyl ether. The solvent of the filtrate was removed *in vacuo* and purified by silica gel column chromatography (hexane/ethyl acetate) to yield the desired compound or crude mixture, which were reacted in the next step of deprotection.

**F. General procedure for deprotection of (2-trimethylsilyl)ethoxy methyl groups (9a-c, 10)**

TBAF in 1 M THF was added to a starting solution of alkyl-substituted triazole (**8a-c**) or non-substituted triazole (**7a**) in dried THF. The mixture was stirred for 24–48 h at 60 °C, and the solution was cooled to room temperature. The mixture was poured into ethyl acetate and water and extracted twice with ethyl acetate. The combined organic layers were dried with $Na_2SO_4$, filtered and concentrated. The residue was purified by silica gel column chromatography (hexane/ethyl acetate) to yield the desired compound.



## *4.2. Chemistry*

### 4.2.1. 4-Iodo-7H-pyrrolo[2,3-d]pyrimidine (4)

The procedure outlined in method A was followed to afford the desired compound with 91.0% yield. $^1$H NMR (400 MHz, DMSO-$d_6$) δ (ppm) *J* (Hz) 12.46 (br s, 1H) 8.39 (s, 1H) 7.63 (d, *J*= 4, 1H) 6.29 (d, *J*= 4, 1H); MS (ESI): $[M]^+$ = 245.6

### 4.2.2 4-Iodo-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidine (5)

The procedure outlined in method B was followed to afford the desired compound with 74.3% yield. $^1$H NMR (400 MHz, $CDCl_3$) δ (ppm) *J* (Hz) 8.53 (s, 1H) 7.40 (d, *J*= 4, 1H) 6.44 (d, *J*= 4, 1H) 5.62 (s, 2H) 3.52 (t, *J*= 8, 2H) 0.91 (t, *J*= 8, 2H) -0.06 (s, 9H); MS (ESI): $[M]^+$ = 377.1

**4.2.3. 4-(Prop-1-yn-1-yl)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidine (6a)**

The procedure outlined in method C-a was followed to afford the desired compound with 81.3% yield. $^1$H NMR (400 MHz, $CDCl_3$) δ (ppm) *J* (Hz) 8.81 (s, 1H) 7.35 (d, *J*= 4, 1H) 6.68 (d, *J*= 4, 1H) 5.63 (s, 2H) 3.51 (t, *J*= 8, 2H) 2.20 (s, 3H) 0.89 (t, *J*= 8, 2H) -0.07 (s, 9H); MS (ESI): $[M]^+$ = 288.4

**4.2.4. 4-(Phenylethynyl)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidine (6b)**

The procedure outlined in method C-b was followed to afford the desired compound with 98% yield. $^1$H NMR (400 MHz, $CDCl_3$) δ (ppm) *J* (Hz) 8.90 (s, 1H) 7.70 (d, *J*= 4, 1H) 7.68 (d, *J*= 4, 1H) 7.43 (d, *J*= 4, 2H) 7.41 (t, *J*= 4, 3H) 6.79 (d, *J*= 4, 1H) 5.67 (s, 2H) 3.56 (t, *J*= 8, 2H) 0.94 (t, *J*= 8, 2H) -0.05 (s, 9H); MS (ESI): [M]+ = 350.1

**4.2.5. 4-(*p*-Tolylethynyl)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidine (6c)**

The procedure outlined in method C-c was followed to afford the desired compound with 57.5% yield. $^1$H NMR (400 MHz, $CDCl_3$) δ (ppm) *J* (Hz) 8.89 (s, 1H) 7.59 (d, *J*= 8, 2H) 7.41 (d, *J*= 4, 1H) 7.23 (d, *J*= 8, 2H) 6.78 (d, *J*= 4, 1H) 5.67 (s, 2H) 3.56 (t, *J*= 8, 2H) 2.41 (s, 3H) 0.94 (t, *J*= 8, 2H) -0.05 (s, 9H); MS (ESI): [M]+ = 363.9

**4.2.6. 4-(5-Methyl-1H-1,2,3-triazol-4-yl)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidine (7a)**

The procedure outlined in method D-a was followed to afford the desired compound with 54.3% yield. $^1$H NMR (400 MHz, $CDCl_3$) δ (ppm) *J* (Hz) 8.96 (s, 1H) 7.43 (d, *J*= 4, 1H) 7.33 (d, *J*= 4, 1H) 5.71 (s, 2H) 3.57 (t, *J*= 8, 2H) 2.87 (s, 3H) 0.93 (t, *J*= 8, 2H) -0.05 (s, 9H); MS (ESI): $[M]^+$ = 332.8

**4.2.7. 4-(5-Phenyl-1H-1,2,3-triazol-4-yl)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidine (7b)**

The procedure outlined in method D-b was followed to afford the desired compound with 76% yield. $^1$H NMR (400 MHz, $CDCl_3$) δ (ppm) *J* (Hz) 8.93 (s, 1H) 7.73 (d, *J*= 4, 1H) 7.72 (d, *J*=8, 1H) 7.40 (d, *J*=8, 3H) 6.67 (d, *J*=4, 1H) 5.69 (s, 2H) 3.58 (t, *J*= 8, 2H) 0.95 (t, *J*= 8, 2H) -0.05 (s, 9H); MS (ESI): $[M]^+$ = 394.0

**4.2.8. 4-(5-(*p*-Tolyl)-1H-1,2,3-triazol-4-yl)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidine (7c)**

The procedure outlined in method D-c was followed to afford the desired compound with 79.9% yield. $^1$H NMR (400 MHz, $CDCl_3$) δ (ppm) *J* (Hz) 8.94 (s, 1H) 7.60 (d, *J*= 8, 2H) 7.35 (d, *J*= 4, 1H) 7.18 (d, *J*= 8, 2H) 6.72 (br s, 1H) 5.69 (s, 2H) 3.58 (t, *J*= 8, 2H) 2.37 (s, 3H) 0.95 (t, *J*= 8, 2H) -0.06 (s, 9H); MS (ESI): $[M]^+$ = 406.9

**4.2.9. 4-(5-Methyl-1H-1,2,3-triazol-4-yl)-7H-pyrrolo[2,3-d]pyrimidine (10)**

Synthesis was according to procedure F. Yield 21.5%; $^1$H NMR (400 MHz, DMSO-$d_6$) δ (ppm) *J* (Hz) 12.13 (br s, 1H) 8.78 (s, 1H) 7.58 (d, *J*= 4, 1H) 7.13 (d, *J*= 4, 1H) 2.72 (s, 3H); MS (ESI) : $[M]^+$ = 200.9

**4.2.10. 4-(1,4-Dimethyl-1H-1,2,3-triazol-5-yl)-7H-pyrrolo[2,3-d]pyrimidine (11)**

Synthesis was according to procedure F. Yield 12.4%; $^1$H NMR (400 MHz, $CDCl_3$) δ (ppm) *J* (Hz) 9.65 (br s, 1H) 9.01 (s, 1H) 7.47 (d, *J*= 4, 1H) 6.50 (d, *J*= 4, 1H) 4.20 (s, 3H) 2.43(s, 3H); MS (ESI): $[M]^+$ = 215.0

**4.2.11. 4-(1-Methyl-4-(p-tolyl)-1H-1,2,3-triazol-5-yl)-7H-pyrrolo[2,3-d]pyrimidine (12)**

Synthesis was according to procedure F. Yield 8.6%; $^1$H NMR (400 MHz, $CDCl_3$) δ (ppm) *J* (Hz) 10.04 (br s, 1H) 9.07 (s, 1H) 7.40 (d, *J*= 8, 2H) 7.22 (d, *J*= 4, 1H) 7.08 (d, *J*= 8, 2H) 5.89 (d, *J*= 4, 1H) 4.14 (s, 3H) 2.05 (s, 3H); MS (ESI): $[M]^+$ = 291.0

**4.2.12. 4-(1-(2-Methoxyethyl)-4-(p-tolyl)-1H-1,2,3-triazol-5-yl)-7H-pyrrolo[2,3-d]pyrimidine (13)**

Synthesis was according to procedure F. Yield 4.0%; $^1$H NMR (400 MHz, $CDCl_3$) δ (ppm) *J* (Hz)

10.14 (br s, 1H) 9.06 (s, 1H) 7.38 (d, *J*= 8, 2H) 7.22 (d, *J*= 4, 1H) 7.06 (d, *J*= 8, 2H) 5.91 (d, *J*= 4, 1H) 4.83 (t, *J*= 8, 2H) 3.76 (t, *J*= 8, 2H) 3.10 (s, 3H) 2.31 (s, 3H); MS (ESI): $[M]^+$ = 334.9

**4.2.13. 4-(1,5-Dimethyl-1H-1,2,3-triazol-4-yl)-7H-pyrrolo[2,3-d]pyrimidine (14)**

Synthesis was according to procedure F. Yield 40.3%; $^1$H NMR (400 MHz, DMSO-$d_6$) δ (ppm) *J* (Hz) 12.15 (br s, 1H) 8.77 (s, 1H) 7.57 (d, *J*= 4, 1H) 7.00 (d, *J*= 4, 1H) 4.20 (s, 3H) 2.63 (s, 3H); MS (ESI): $[M]^+$ = 215.0

**4.2.14. 4-(1-Methyl-5-(p-tolyl)-1H-1,2,3-triazol-4-yl)-7H-pyrrolo[2,3-d]pyrimidine (15)**

Synthesis was according to procedure F. Yield 13.5%; $^1$H NMR (400 MHz, $CDCl_3$) δ (ppm) *J* (Hz) 10.37 (br s, 1H) 8.69 (s, 1H) 7.36 (d, *J*= 8, 2H) 7.35 (d, *J*= 4, 1H) 7.32 (d, *J*= 8, 2H) 7.20 (d, *J*= 4, 1H) 4.03 (s, 3H) 2.45 (s, 3H); MS (ESI): $[M]^+$ = 290.9

**4.2.15. 4-(5-Methyl-1-vinyl-1H-1,2,3-triazol-4-yl)-7H-pyrrolo[2,3-d]pyrimidine (16)**

Synthesis was according to procedure F. Yield 72.3%; $^1$H NMR (400 MHz, $CDCl_3$) δ (ppm) *J* (Hz) 9.13 (br s, 1H) 8.89 (s, 1H) 7.49 (d, *J*= 4, 1H) 7.39 (d, *J*= 4, 1H) 7.20 (dd, $^1J$= 16 $^2J$= 12, 1H) 6.21 (d, *J*= 12, 1H) 5.39 (d, *J*= 12, 1H) 2.94 (s, 3H); MS (ESI): $[M]^+$ = 227.3

**4.2.16. 4-(1-Allyl-5-methyl-1H-1,2,3-triazol-4-yl)-7H-pyrrolo[2,3-d]pyrimidine (17)**

Synthesis was according to procedure F. Yield 77.6%; $^1$H NMR (400 MHz, $CDCl_3$) δ (ppm) *J* (Hz) 9.66 (br s, 1H) 8.90 (s, 1H) 7.53 (dd, $^1J$= 8 $^2J$= 4, 1H) 7.41 (dd, $^1J$= 8 $^2J$= 4, 1H) 6.78 (d, *J*= 4, 1H) 6.08 (m, 1H) 2.84 (s, 3H) 1.95 (d, *J*= 4, 2H); MS (ESI): $[M]^+$ = 241.4

**4.2.17. 4-(5-Methyl-1-(2-methylallyl)-1H-1,2,3-triazol-4-yl)-7H-pyrrolo[2,3-d]pyrimidine (18)**

Synthesis was according to procedure F. Yield 30.9%; $^1$H NMR (400 MHz, $CDCl_3$) δ (ppm) *J* (Hz) 11.20 (br, 1H) 8.92 (s, 1H) 7.50 (d, *J*= 4, 1H) 7.46 (d, *J*= 4, 1H) 5.03 (s, 1H) 4.96 (s, 1H) 4.73(s, 1H) 2.83(s, 3H) 1.78(s, 3H); MS (ESI): $[M]^+$ = 255.2

**4.2.18. 4-(5-Methyl-1-(3-methylbut-2-en-1-yl)-1H-1,2,3-triazol-4-yl)-7H-pyrrolo[2,3-d]pyrimidine (19)**

Synthesis was according to procedure F. Yield 96.9%; $^1$H NMR (400 MHz, $CDCl_3$) δ (ppm) *J* (Hz) 9.64 (br s, 1H) 8.88 (s, 1H) 7.48 (d, *J*= 4, 1H) 7.38 (d, *J*= 4, 1H) 5.38 (t, *J*= 8, 1H) 5.01 (d, *J*= 4, 2H) 2.84 (s, 3H) 1.87 (s, 3H) 1.79 (s, 3H); MS (ESI): $[M]^+$ = 269.4

**4.2.19. 2-(5-Methyl-4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-1,2,3-triazol-1-yl)ethan-1-amine (20)**

Synthesis was according to procedure F. Yield 18.0 %; $^1$H NMR (400 MHz, $CD_3OD$) δ (ppm) *J* (Hz) 8.77 (s, 1H) 7.49 (d, *J*= 4, 1H) 7.23 (d, *J*= 4, 1H) 4.56 (t, *J*= 8, 2H) 3.31 (t, *J*= 8, 2H) 2.84 (s, 3H); MS (ESI): $[M]^+$ = 244.4

**4.2.20. 4-(1-(2-Methoxyethyl)-5-methyl-1H-1,2,3-triazol-4-yl)-7H-pyrrolo[2,3-d]pyrimidine (21)**

Synthesis was according to procedure F. Yield 58.6%; $^1$H NMR (400 MHz, $CDCl_3$) δ (ppm) *J* (Hz) 9.88 (br s, 1H) 8.90 (s, 1H) 7.49 (d, *J*= 4, 1H) 7.40 (d, *J*= 4, 1H) 4.54 (t, *J*= 8, 2H) 3.88 (t, *J*= 8, 2H) 3.34 (s, 3H) 2.90 (s, 3H); MS (ESI): $[M]^+$ = 259.4

**4.2.21. 4-(1-(2-Methoxyethyl)-5-(p-tolyl)-1H-1,2,3-triazol-4-yl)-7H-pyrrolo[2,3-d]pyrimidine (22)**

Synthesis was according to procedure F. Yield 31.8%; $^1$H NMR (400 MHz, $CDCl_3$) δ (ppm) *J* (Hz) 9.78 (br s, 1H) 8.67 (s, 1H) 7.40 (d, *J*= 8, 2H) 7.35 (d, *J*= 4, 1H) 7.31 (d, *J*= 8, 2H) 7.24 (d, *J*= 4, 1H) 4.47 (t, *J*= 8, 2H) 3.88 (t, *J*= 8, 2H) 3.29 (s, 3H) 2.45 (s, 3H); MS (ESI): $[M]^+$ = 335.0

**4.2.22. 4-(5-Methyl-1-(4,4,4-trifluorobutyl)-1H-1,2,3-triazol-4-yl)-7H-pyrrolo[2,3-d]pyrimidine (23)**

Synthesis was according to procedure F. Yield 15.6%; $^1$H NMR (400 MHz, $CDCl_3$) δ (ppm) *J* (Hz) 9.55 (br, 1H) 8.90 (s, 1H) 7.49 (d, *J*= 4, 1H) 7.40 (d, *J*= 4, 1H) 4.48 (t, *J*= 8, 2H) 2.90 (s, 3H) 2.29 (m, 4H); MS (ESI): $[M]^+$ = 311.2

**4.2.23. 3-(5-Methyl-4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-1,2,3-triazol-1-yl)propan-1-ol (24)**

Synthesis was according to procedure F. Yield 36.6%; $^1$H NMR (400 MHz, $CD_3OD$) δ (ppm) *J* (Hz) 8.75 (s, 1H) 7.46 (d, *J*= 4, 1H) 7.20 (d, *J*= 4, 1H) 4.2 (t, *J*= 8, 2H) 3.63 (t, *J*= 8, 2H) 2.88 (s, 3H) 2.16 (t, *J*= 8, 2H); MS (ESI): $[M]^+$ = 259.5

**4.2.24. 5-(5-Methyl-4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-1,2,3-triazol-1-yl)pentan-1-ol (25)**

Synthesis was according to procedure F. Yield 47.9%; $^1$H NMR (400 MHz, $CD_3OD$) δ (ppm) *J* (Hz) 8.76 (s, 1H) 7.48 (d, *J*= 4, 1H) 7.21 (d, *J*= 4, 1H) 4.46 (t, *J*= 8, 2H) 3.58 (t, *J*= 8, 2H) 2.81 (s, 3H) 1.99 (m, 2H) 1.65 (m, 2H) 1.46 (m, 2H); MS (ESI) : $[M]^+$ = 287.0

**4.2.25. 4-(5-Methyl-4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-1,2,3-triazol-1-yl)butanenitrile (26)**

Synthesis was according to procedure F. Yield 12.1%; $^1$H NMR (400 MHz, $CD_3OD$) δ (ppm) *J* (Hz) 12.09 (br s, 1H) 8.74 (s, 1H) 7.55 (d, *J*= 4, 1H) 7.15 (d, *J*= 4, 1H) 4.45 (t, *J*= 8, 2H) 2.79 (s, 3H) 2.59 (t, *J*= 8, 2H) 2.15 (quint, *J*= 8, 2H); MS (ESI) : $[M]^+$ = 268.3

**4.2.26. 5-(5-Methyl-4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-1,2,3-triazol-1-yl)pentanenitrile (27)**

Synthesis was according to procedure F. Yield 10.5%; $^1$H NMR (400 MHz, $CD_3OD$) δ (ppm) *J* (Hz) 12.08 (br s, 1H) 8.74 (s, 1H) 7.54 (d, *J*= 4, 1H) 7.15 (d, *J*= 4, 1H) 4.42 (t, *J*= 8, 2H) 2.78 (s, 3H) 2.56 (t, *J*= 8, 2H) 1.93 (m, 2H) 1.60 (m, 2H); MS (ESI): $[M]^+$ = 282.5

**4.2.27. 5-(5-Phenyl-4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-1,2,3-triazol-1-yl)pentanenitrile (28)**

Synthesis was according to procedure F. Yield 9.4%; $^1$H NMR (400 MHz, $CDCl_3$) δ (ppm) *J* (Hz) 9.62 (br s, 1H) 8.63 (s, 1H) 7.55 (m, 3H) 7.46 (d, *J*= 4, 1H) 7.44 (m, 1H) 7.43 (d, *J*= 4, 1H) 7.26 (m, 1H) 4.41 (t, *J*= 8, 2H) 2.35 (t, *J*= 8, 2H) 2.04 (m, 2H) 1.72 (m, 2H); MS (ESI): $[M]^+$ = 343.9

**4.2.28. 5-(4-(7H-Pyrrolo[2,3-d]pyrimidin-4-yl)-5-(*p*-tolyl)-1H-1,2,3-triazol-1-yl)pentanenitrile (29)**

Synthesis was according to procedure F. Yield 15.2%; $^1$H NMR (400 MHz, $CDCl_3$) δ (ppm) *J* (Hz) 9.65 (br s, 1H) 8.66 (s, 1H) 7.38 (d, *J*= 8, 2H) 7.26 (d, *J*= 4, 1H) 7.20 (d, *J*= 8, 2H) 7.07 (d, *J*= 4, 1H) 4.40 (t, *J*= 8, 2H) 2.47 (s, 3H) 2.07 (m, 2H) 1.71 (m, 2H) 0.90 (m, 2H); MS (ESI): $[M]^+$ = 357.9

**4.2.29. 6-(5-Methyl-4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-1,2,3-triazol-1-yl)hexanenitrile (30)**

Synthesis was according to procedure F. Yield 11.9%; $^1$H NMR (400 MHz, $CDCl_3$) δ (ppm) *J* (Hz) 9.07 (br s, 1H) 8.88 (s, 1H) 7.48 (d, *J*= 4, 1H) 7.38 (d, *J*= 4, 1H) 4.42 (t, *J*= 8, 2H) 2.88 (s, 3H) 2.40 (t,

*J*= 8, 2H) 2.04 (m, 2H) 1.80 (m, 2H) 1.58 (m, 2H); MS (ESI): $[M]^+$ = 296.5

**4.2.30. 4-(1-(Cyclopropylmethyl)-5-methyl-1H-1,2,3-triazol-4-yl)-7H-pyrrolo[2,3-d]pyrimidine (31)**

Synthesis was according to procedure F. Yield 11.3%; $^1$H NMR (400 MHz, $CDCl_3$) δ (ppm) *J* (Hz) 9.94 (br s, 1H) 8.89 (s, 1H) 7.51 (d, *J*= 4, 1H) 7.40 (d, *J*= 4, 1H) 4.27 (d, *J*= 8, 2H) 2.91 (s, 3H) 1.38 (m, 1H) 0.71 (q, *J*= 8, 2H) 0.52 (m, *J*= 8, 2H); MS (ESI): $[M]^+$ = 256.1

**4.2.31. 4-(1-(Cyclobutylmethyl)-5-methyl-1H-1,2,3-triazol-4-yl)-7H-pyrrolo[2,3-d]pyrimidine (32)**

Synthesis was according to procedure F. Yield 11.3%; $^1$H NMR (400 MHz, $CDCl_3$) δ (ppm) *J* (Hz) 9.94 (br s, 1H) 8.89 (s, 1H) 7.51 (d, *J*= 4, 1H) 7.40 (d, *J*= 4, 1H) 4.27 (d, *J*= 8, 2H) 2.91 (s, 3H) 1.38 (m, 1H) 0.71 (q, *J*= 8, 2H) 0.52 (m, *J*= 8, 2H); MS (ESI): $[M]^+$ = 269.1

**4.2.32. 4-(1-(Cyclopentylmethyl)-5-methyl-1H-1,2,3-triazol-4-yl)-7H-pyrrolo[2,3-d]pyrimidine (33)**

Synthesis was according to procedure F. Yield 7.6%; $^1$H NMR (400 MHz, $CDCl_3$) δ (ppm) *J* (Hz) 9.46 (br s, 1H) 8.88 (s, 1H) 7.50 (d, *J*= 4, 1H) 7.38 (d, *J*= 4, 1H) 4.30 (d, *J*= 8, 2H) 2.88 (s, 3H) 1.80 (m, 2H) 1.72 (m, 2H) 1.42 (m, 2H) 0.99 (m, 2H); MS (ESI): $[M]^+$ = 283.2

**4.2.33. 4-(2-(Cyclohexylmethyl)-5-methyl-2H-1,2,3-triazol-4-yl)-7H-pyrrolo[2,3-d]pyrimidine (34)**

Synthesis was according to procedure F. Yield 14.2%; $^1$H NMR (400 MHz, $CDCl_3$) δ (ppm) *J* (Hz) 9.38 (br s, 1H) 8.88 (s, 1H) 7.51 (d, *J*= 4, 1H) 7.38 (d, *J*= 4, 1H) 4.21 (d, *J*= 4, 2H) 2.86 (s, 3H) 2.64 (m, 1H) 2.03 (m, 2H) 1.76 (m, 2H) 1.55 (m, 2H) 1.13 (m, 2H) 0.88 (m, 2H); MS (ESI): $[M]^+$ = 297.1

**4.2.34. 4-(2,5-Dimethyl-2H-1,2,3-triazol-4-yl)-7H-pyrrolo[2,3-d]pyrimidine (35)**

Synthesis was according to procedure F. Yield 18.4%; $^1$H NMR (400 MHz, DMSO-$d_6$) δ (ppm) *J* (Hz) 12.09 (br s, 1H) 8.73 (s, 1H) 7.53 (d, *J*= 4, 1H) 7.14 (d, *J*= 4, 1H) 4.00 (s, 3H) 2.75 (s, 3H); MS (ESI): $[M]^+$ = 215.1

**4.2.35. 4-(2-Methyl-5-(p-tolyl)-2H-1,2,3-triazol-4-yl)-7H-pyrrolo[2,3-d]pyrimidine (36)**

Synthesis was according to procedure F. Yield 27.2%; $^{1}$H NMR (400 MHz, $CDCl_3$) δ (ppm) *J* (Hz) 10.52 (br s, 1H) 8.94 (s, 1H) 7.63 (d, *J*= 8, 2H) 7.34 (d, *J*= 4, 1H) 7.19 (d, *J*= 8, 2H) 6.67 (d, *J*= 4, 1H) 4.38 (s, 3H) 2.38 (s, 3H); MS (ESI): $[M]^+$ = 291.0

**4.2.36. 4-(5-Methyl-2-vinyl-2H-1,2,3-triazol-4-yl)-7H-pyrrolo[2,3-d]pyrimidine (37)**

Synthesis was according to procedure F. Yield 15.1%; $^{1}$H NMR (400 MHz, $CDCl_3$) δ (ppm) *J* (Hz) 9.32 (br s, 1H) 8.95 (s, 1H) 7.40 (d, *J*= 4, 1H) 7.38 (dd, $^{1}J$= 8 $^{2}J$= 4, 1H) 7.38 (dd, $^{1}J$= 16 $^{2}J$= 8, 1H) 7.26 (dd, $^{1}J$= 8 $^{2}J$= 4, 1H) 6.06 (d, *J*= 16, 1H) 5.14 (d, *J*= 16, 1H) 2.84 (s, 3H); MS (ESI): $[M]^+$ = 227.3

**4.2.37. 4-(2-Allyl-5-methyl-2H-1,2,3-triazol-4-yl)-7H-pyrrolo[2,3-d]pyrimidine (38)**

Synthesis was according to procedure F. Yield 83.2%; $^{1}$H NMR (400 MHz, $CDCl_3$) δ (ppm) *J* (Hz) 9.16 (br s, 1H) 8.93 (s, 1H) 7.38 (d, *J*= 4, 1H) 7.25 (d, *J*= 4, 1H) 7.18 (m, 1H) 7.40 (m, 5H) 7.14 (m, 1H) 6.60 (dd, $^{1}J$= 8 $^{2}J$= 4, 1H) 2.81 (s, 3H) 1.95 (d, *J*= 4, 2H). $^{13}$C NMR (DMSO-$d_6$) δ (ppm) 152.74, 151.14, 149.37, 144.92, 142.95, 142.62, 127.92, 114.70, 101.93, 60.74, 20.27, 12.82 ; MS (ESI): $[M]^+$ = 241.4

**4.2.38. 4-(5-Methyl-2-(2-methylallyl)-2H-1,2,3-triazol-4-yl)-7H-pyrrolo[2,3-d]pyrimidine (39)**

Synthesis was according to procedure F. Yield 13.9%; $^{1}$H NMR (400 MHz, $CDCl_3$) δ (ppm) *J* (Hz) 10.79 (br s, 1H) 8.97 (s, 1H) 7.44 (d, *J*= 4, 1H) 7.22 (d, *J*= 4, 1H) 5.06 (d, *J*= 32, 2H) 5.05 (s, 1H) 2.80 (s, 3H) 1.78 (s, 3H) $^{13}$C NMR (DMSO-$d_6$) δ (ppm) 152.82, 1521.12, 150.26, 142.41, 140.25, 135.52, 127.44, 117.81, 114.31, 113.10, 102.39, 53.18, 20.23, 10.42; MS (ESI): $[M]^+$ = 255.2 HRMS (FAB) found for $C_{13}H_{14}N_6$ $[M + H]^+$ 255.1358.

**4.2.39. 4-(5-Methyl-2-(3-methylbut-2-en-1-yl)-2H-1,2,3-triazol-4-yl)-7H-pyrrolo[2,3-d]pyrimidine (40)**

Synthesis was according to procedure F. Yield 59.5%; $^{1}$H NMR (400 MHz, $CDCl_3$) δ (ppm) *J* (Hz)

9.85 (br s, 1H) 8.94 (s, 1H) 7.40 (d, *J*= 4, 1H) 7.22 (d, *J*= 4, 1H) 5.60 (t, *J*= 8, 1H) 5.10 (d, *J*= 4, 2H) 2.78 (s, 3H) 1.88 (s, 3H) 1.82 (s, 3H) $^{13}$C NMR (DMSO-$d_6$) δ (ppm) 152.75, 151.15, 149.48, 144.73, 142.63, 138.56, 127.85, 118.61, 114.44, 102.00, 52.91, 25.88, 18.49, 12.85 ; MS (ESI): $[M]^+$ = 269.3

**4.2.40. 2-(4-Methyl-5-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-2H-1,2,3-triazol-2-yl)ethan-1-amine (41)**

Synthesis was according to procedure F. Yield 21.2%; $^1$H NMR (400 MHz, $CD_3OD$) δ (ppm) *J* (Hz) 8.77 (s, 1H) 7.47 (d, *J*= 4, 1H) 7.13 (d, *J*= 4, 1H) 4.61 (t, *J*= 8, 2H) 3.31 (t, *J*= 8, 2H) 2.71 (s, 3H); MS (ESI): $[M]^+$ = 244.4

**4.2.41. Tert-butyl(2-(4-methyl-5-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-2H-1,2,3-triazol-2-yl)ethyl)carbamate (42)**

Synthesis was according to procedure F. Yield 37.4%; $^1$H NMR (400 MHz, $CD_3OD$) δ (ppm) *J* (Hz) 8.76 (s, 1H) 7.46 (d, *J*= 4, 1H) 7.16 (d, *J*= 4, 1H) 4.58 (t, *J*= 8, 2H) 3.64 (t, *J*= 8, 2H) 2.70 (s, 3H) 1.36 (s, 9H); MS (ESI): $[M]^+$ = 344.3

**4.2.42. 4-(2-(2-Methoxyethyl)-5-methyl-2H-1,2,3-triazol-4-yl)-7H-pyrrolo[2,3-d]pyrimidine (43)**

Synthesis was according to procedure F. Yield 71.4%; $^1$H NMR (400 MHz, $CDCl_3$) δ (ppm) *J* (Hz) 10.12 (br s, 1H) 8.95 (s, 1H) 7.40 (d, *J*= 4, 1H) 7.22 (d, *J*= 4, 1H) 4.67 (t, *J*= 8, 2H) 4.00 (t, *J*= 8, 2H) 3.40 (s, 3H) 2.79 (s, 3H) $^{13}$C NMR (DMSO-$d_6$) δ (ppm) 152.75, 151.14, 149.43, 144.70, 142.73, 127.84, 114.48, 102.01, 70.23, 58.43, 54.74, 12.85; MS (ESI): $[M]^+$ = 259.2

**4.2.43. 4-(2-(2-Methoxyethyl)-5-(*p*-tolyl)-2H-1,2,3-triazol-4-yl)-7H-pyrrolo[2,3-d]pyrimidine (44)**

Synthesis was according to procedure F. Yield 50.7%; $^1$H NMR (400 MHz, $CDCl_3$) δ (ppm) *J* (Hz) 10.72 (br s, 1H) 8.94 (s, 1H) 7.66 (d, *J*= 8, 2H) 7.35 (d, *J*= 4, 1H) 7.19 (d, *J*= 8, 2H) 6.70 (d, *J*= 4, 1H) 4.78 (t, *J*= 8, 2H) 4.07 (t, *J*= 8, 2H) 3.42 (s, 3H) 2.38 (s, 3H); MS (ESI): $[M]^+$ = 335.2

**4.2.44. 4-(5-Methyl-2-(4,4,4-trifluorobutyl)-2H-1,2,3-triazol-4-yl)-7H-pyrrolo[2,3-d]pyrimidine (45)**

Synthesis was according to procedure F. Yield 52.7%; $^1$H NMR (400 MHz, $CDCl_3$) δ (ppm) *J* (Hz)

10.46 (br s, 1H) 8.97 (s, 1H) 7.45 (d, *J*= 4, 1H) 7.19 (d, *J*= 4, 1H) 4.60 (t, *J*= 8, 2H) 2.80 (s, 3H) 2.37 (m, 2H) 2.27 (m 2H) $^{13}$C NMR (DMSO-$d_6$) δ (ppm) 152.73, 151.13, 149.33, 144.91, 142.87, 127.94, 126.53, 114.49, 101.85, 53.43, 30.29, 22.39, 12.85; MS (ESI): $[M]^+$ = 311.5 HRMS (FAB) found for $C_{13}H_{13}F_3N_6$ $[M + H]^+$ 311.1232.

**4.2.45. 3-(4-Methyl-5-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-2H-1,2,3-triazol-2-yl)propan-1-ol (46)**

Synthesis was according to procedure F. Yield 86.9%; $^1$H NMR (400 MHz, $CD_3OD$) δ (ppm) *J* (Hz) 8.75 (s, 1H) 7.45 (d, *J*= 4, 1H) 7.12 (d, *J*= 4, 1H) 4.59 (t, *J*= 8, 2H) 3.66 (t, *J*= 8, 2H) 2.69 (s, 3H) 2.24 (t, *J*= 8, 2H) $^{13}$C NMR (DMSO-$d_6$) δ (ppm) 152.73, 151.14, 149.48, 144.56, 142.56, 127.82, 114.45, 102.02, 58.18, 52.24, 32.90, 12.85; MS (ESI): $[M]^+$ = 259.3

**4.2.46. 5-(4-Methyl-5-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-2H-1,2,3-triazol-2-yl)pentan-1-ol (47)**

Synthesis was according to procedure F. Yield 25.4%; $^1$H NMR (400 MHz, $CD_3OD$) δ (ppm) *J* (Hz) 8.77 (s, 1H) 7.47 (d, *J*= 4, 1H) 7.13 (d, *J*= 4, 1H) 4.53 (t, *J*= 8, 2H) 3.57 (t, *J*= 8, 2H) 2.70 (s, 3H) 2.08 (m, 2H) 1.62 (m, 2H) 1.45 (m, 2H); MS (ESI): $[M]^+$ = 287.3

**4.2.47. Ethyl 5-(4-methyl-5-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-2H-1,2,3-triazol-2-yl)pentanoate (48)**

Synthesis was according to procedure F. Yield 24.3%; $^1$H NMR (400 MHz, $CDCl_3$) δ (ppm) *J* (Hz) 9.81 (br s, 1H) 8.94 (s, 1H) 7.40 (d, *J*= 4, 1H) 7.21 (d, *J*= 4, 1H) 4.51 (t, *J*= 8, 2H) 4.16 (q, *J*= 8, 2H) 2.78 (s, 3H) 2.39 (t, *J*= 8, 2H) 2.14 (m, 2H) 1.78 (m, 2H) 1.27 (t, *J*= 8, 2H); MS (ESI): $[M]^+$ = 329.3

**4.2.48. 4-(4-Methyl-5-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-2H-1,2,3-triazol-2-yl)butanenitrile (49)**

Synthesis was according to procedure F. Yield 64.5%; $^1$H NMR (400 MHz, $CD_3OD$) δ (ppm) *J* (Hz) 12.15 (br s, 1H) 8.78 (s, 1H) 7.57 (d, *J*= 4, 1H) 7.02 (d, *J*= 4, 1H) 4.56 (t, *J*= 8, 2H) 2.64 (s, 3H) 2.57 (t, *J*= 8, 2H) 2.23 (quint, *J*= 8, 2H) $^{13}$C NMR (DMSO-$d_6$) δ (ppm) 152.76, 151.12, 149.33, 144.99, 142.95, 127.90, 120.28, 114.51, 102.00, 53.51, 25.60, 14.49, 12.87; MS (ESI): $[M]^+$ = 269.3 HRMS (FAB) found for $C_{13}H_{13}N_7$ $[M + H]^+$ 268.1311.

**4.2.49. 5-(4-Methyl-5-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-2H-1,2,3-triazol-2-yl)pentanenitrile (50)**

Synthesis was according to procedure F. Yield 39.5%; $^{1}$H NMR (400 MHz, $CDCl_3$) δ (ppm) *J* (Hz) 10.20 (br s, 1H) 8.95 (s, 1H) 7.43 (d, *J*= 4, 1H) 7.19 (d, *J*= 4, 1H) 4.58 (t, *J*= 8, 2H) 2.79 (s, 3H) 2.46 (t, *J*= 8, 2H) 2.27 (m, 2H) 1.81 (m, 2H) $^{13}$C NMR (DMSO-$d_6$) δ (ppm) 152.75, 151.13, 149.41, 144.74, 142.71, 127.82, 121.25, 120.95, 114.48, 101.99, 54.07, 28.66, 22.61, 16.25, 12.85; MS (ESI): $[M]^+$ = 282.3 HRMS (FAB) found for $C_{14}H_{15}N_7$ $[M + H]^+$ 282.1467.

**4.2.50. 5-(4-Phenyl-5-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-2H-1,2,3-triazol-2-yl)pentanenitrile (51)**

Synthesis was according to procedure F. Yield 37.5%; $^{1}$H NMR (400 MHz, $CDCl_3$) δ (ppm) *J* (Hz) 10.19 (br s, 1H) 8.94 (s, 1H) 7.76 (m,2H) 7.40 (m, 3H) 7.36 (d, *J*= 4, 1H) 6.66 (d, *J*= 4, 1H) 4.69 (t, *J*= 8, 2H) 2.49 (t, *J*= 8, 2H) 2.32 (m, 2H) 1.87 (m, 2H); MS (ESI): $[M]^+$ = 344.1

**4.2.51. 5-(4-(7H-Pyrrolo[2,3-d]pyrimidin-4-yl)-5-(*p*-tolyl)-2H-1,2,3-triazol-2-yl)pentanenitrile (52)**

Synthesis was according to procedure F. Yield 25.6%; $^{1}$H NMR (400 MHz, $CDCl_3$) δ (ppm) *J* (Hz) 9.97 (br s, 1H) 8.93 (s, 1H) 7.65 (d, *J*= 8,2H) 7.34 (d, *J*= 4, 1H) 7.20 (d, *J*= 8, 2H) 6.68 (d, *J*= 4, 1H) 4.67 (t, *J*= 8, 2H) 2.48 (t, *J*= 8, 2H) 2.39 (s, 3H) 2.29 (m, 2H) 1.87 (m, 2H); MS (ESI): $[M]^+$ = 358.1

**4.2.52. 6-(4-Methyl-5-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-2H-1,2,3-triazol-2-yl)hexanenitrile (53)**

Synthesis was according to procedure F. Yield 36.9%; $^{1}$H NMR (400 MHz, $CDCl_3$) δ (ppm) *J* (Hz) 9.95 (br s, 1H) 8.94 (s, 1H) 7.41 (d, *J*= 4, 1H) 7.19 (d, *J*= 4, 1H) 4.54 (t, *J*= 8, 2H) 2.78 (s, 3H) 2.39 (t, *J*= 8, 2H) 2.14 (m, 2H) 1.79 (m, 2H) 1.55 (m, 2H) $^{13}$C NMR (DMSO-$d_6$) δ (ppm) 152.73, 151.14, 149.46, 144.61, 142.59, 127.84, 121.11, 114.47, 102.02, 54.68, 28.79, 25.68, 24.74, 16.52, 12.86; MS (ESI): $[M]^+$ = 296.3

**4.2.53. 4-(2-(Cyclopropylmethyl)-5-methyl-2H-1,2,3-triazol-4-yl)-7H-pyrrolo[2,3-d]pyrimidine (54)**

Synthesis was according to procedure F. Yield 35.7%; $^{1}$H NMR (400 MHz, $CDCl_3$) δ (ppm) *J* (Hz)

10.48 (br s, 1H) 8.96 (s, 1H) 7.42 (d, *J*= 4, 1H) 7.24 (d, *J*= 4, 1H) 4.36 (d, *J*= 8, 2H) 2.80 (s, 3H) 1.52 (m, 1H) 0.71 (q, *J*= 8, 2H) 0.53 (m, *J*= 8, 2H) $^{13}$C NMR (DMSO-$d_6$) δ (ppm) 152.74, 151.16, 149.53, 144.56, 142.64, 127.81, 114.48, 102.03, 59.36, 12.83, 11.49, 4.19; MS (ESI): $[M]^+$ = 255.1 HRMS (FAB) found for $C_{13}H_{14}N_6$ $[M + H]^+$ 255.1358.

**4.2.54. 4-(2-(Cyclobutylmethyl)-5-methyl-2H-1,2,3-triazol-4-yl)-7H-pyrrolo[2,3-d]pyrimidine (55)**

Synthesis was according to procedure F. Yield 28.9%; $^1$H NMR (400 MHz, $CDCl_3$) δ (ppm) *J* (Hz) 10.89 (br s, 1H) 8.96 (s, 1H) 7.44 (d, *J*= 4, 1H) 7.23 (d, *J*= 4, 1H) 4.51 (d, *J*= 8, 2H) 3.07 (m, 1H) 2.78 (s, 3H) 2.19 (m, 2H) 1.96 (m, 2H) $^{13}$C NMR (DMSO-$d_6$) δ (ppm) 152.73, 151.15, 149.49, 144.47, 142.59, 127.84, 114.47, 101.94, 59.51,39.43, 35.34, 25.73, 18.25, 12.82; MS (ESI): $[M]^+$ = 269.1 HRMS (FAB) calculated for $C_{14}H_{14}N_6$ $[M + H]^+$ 269.1515.

**4.2.55. 4-(2-(Cyclopentylmethyl)-5-methyl-2H-1,2,3-triazol-4-yl)-7H-pyrrolo[2,3-d]pyrimidine (56)**

Synthesis was according to procedure F. Yield 18.6%; $^1$H NMR (400 MHz, $CDCl_3$) δ (ppm) *J* (Hz) 10.10 (br s, 1H) 8.95 (s, 1H) 7.40 (d, *J*= 4, 1H) 7.23 (d, *J*= 4, 1H) 4.42 (d, *J*= 8, 2H) 2.78 (s, 3H) 2.68 (m, 1H) 1.82 (m, 2H) 1.70 (m, 2H) 1.67 (m, 2H) 1.43 (m, 2H) $^{13}$C NMR (DMSO-$d_6$) δ (ppm) 152.72, 151.15, 14951, 144.45, 142.51, 127.85, 114.47, 101.95, 59.39, 40.68, 39.63, 30.13, 25.14, 12.83; MS (ESI): $[M]^+$ = 283.2 HRMS (FAB) found for $C_{15}H_{18}N_6$ $[M + H]^+$ 283.1671.

**4.2.56. 4-(2-(Cyclohexylmethyl)-5-methyl-2H-1,2,3-triazol-4-yl)-7H-pyrrolo[2,3-d]pyrimidine (57)**

Synthesis was according to procedure F. Yield 29.0%; $^1$H NMR (400 MHz, $CDCl_3$) δ (ppm) *J* (Hz) 11.18 (br s, 1H) 8.96 (s, 1H) 7.44 (d, *J*= 4, 1H) 7.22 (d, *J*= 4, 1H) 4.33 (d, *J*= 4, 2H) 2.79 (s, 3H) 2.18 (m, 1H) 1.77 (m, 4H) 1.32 (m, 4H) 1.14 (m, 2H); MS (ESI): $[M]^+$ = 297.1

***4.3. Assays***

#### 4.3.1. JAK1 enzyme assay

JAK1 (h) was incubated with 20 mM Tris/HCl pH 7.5, 0.2 mM EDTA, 500 μM GEEPLYWSFPAKKK, 10 mM Mg-acetate and [9-33P-ATP] (specific activity approx. 500 cpm/pmol, concentration as required). The reaction was initiated by the addition of the Mg-ATP mixture. After incubation for 40 minutes at room temperature, the reaction was stopped by the addition of 3% phosphoric acid solution. A 10 μL aliquot of the reaction was spotted onto a P30 filtermat and washed three times with 75 mM phosphoric acid for 5 minutes and once with methanol prior to drying and scintillation counting.

#### 4.3.2. JAK2 enzyme assay

Inhibition of the kinase activity of the recombinant JAK2 protein was measured using homogeneous time-resolved fluorescence (HTRF) assays. Briefly, the assay is based on the phosphorylation of peptide substrates in the presence of ATP, and the resulting phosphorylated substrates are detected by TR-FRET (Time Resolved-Fluorescence Resonance Energy Transfer). The recombinant protein containing the kinase domain was purchased from Millipore (Billerica, MA). The optimal enzyme, ATP, and substrate concentrations were established for the enzyme using the HTRF KinEASE kit (Cisbio, France) according to the manufacturer's instruction. The assays consisted of the enzyme mixed with serially diluted compounds and peptide substrates in kinase reaction buffer (50 mM HEPES (pH 7.0), 0.1 mM orthovanadate, 0.01% BSA, 0.02% NaN3). For JAK2, 10 mM ATP, 5 mM MgCl2, and 1 mM DTT were used. The TR-FRET signal was measured using an EnVision multi-label reader (Perkin Elmer, Waltham, MA). IC50 values were calculated by nonlinear regression using Prism version 5.01 (GraphPad, La Jolla, CA).

#### 4.3.3. JAK3 enzyme assay

The procedure was the same as that used for the JAK2 enzyme assay.

#### 4.3.4. JAK2V617F mutant enzyme assay

A 2X JAK2 JH1 JH2 V617F / Tyr 06 mixture was prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM $MgCl_2$, 1 mM EGTA. The final 10 µL kinase reaction consisted of 7.38 - 100 ng JAK2 JH1 JH2 V617F and 2 µM Tyr 06 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM $MgCl_2$, 1 mM EGTA. After 1 h of incubation, 5 µL of a 1:128 dilution of Development Reagent A was added.

### *4.4. Cell proliferation assay*

#### 4.4.1. Anti-proliferative activity assay by WST in HEL 92.1.7 cells

The anti-proliferative activity of the triazole compounds was determined by an WST-1 assay. HEL 92.1.7 cells (human erythroleukemia cell line) were seeded into 96-well plates at a density of 5 x 104 cells/well in 100 µL of medium; each compound was added to a final concentration of 10 µM and incubated for 3 days at 37°C in a humidified atmosphere of 5% CO2-95% air. After incubation, 10 µL of tetrazolium salt reagent (WST-1 reagent) was added each well and incubated for 4 h at 37°C in a humidified atmosphere of 5% CO2-95% air. The absorbance of the medium was measured using microplate reader at 450 nm. The data are expressed as the percentage of viable cells compared to the control group.

#### 4.4.2. Cytotoxicity effect of HCC827 wild type and gefitinib-resistant HCC827 cell lines

The cytotoxicity effect was determined by the MTT assay. A total of $2x10^3$ cells in 200 µL of medium in 96-well plates were treated with various concentrations of **54** and Ruxolitinib for 72 h at 37 °C. Viable cells were stained with MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (2 mg/mL) for 4 h. The medium was removed, and the water-insoluble formazan was solubilized by adding 200 µL of DMSO to each well. Absorbance was measured at 570 nm using a microtiter plate

reader (Berthold Technologies, Bad Wildbad, Germany).

### *4.5. Western blotting*

HCC827 cells were treated with various concentration of **54** for 24 h. Thereafter, the cells were harvested and lysed with the addition of EBC lysis buffer containing 20 mM Tris-Cl (pH 7.5), 1% Triton X-100, 137 mM sodium chloride, 10% glycerol, 2 mM EDTA, 1 mM sodium orthovanadate, 25 mM B-glycerophosphate, 2 mM sodium pyrophosphate, 1 mM phenylmethylsulfonylfluoride, and 1 μg/mL leupeptin. The cell lysates were centrifuged at 13,000 x g for 15 min to remove the insoluble materials. A 50 μg sample of the cell lysate was resolved by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE, Bio-Rad), and the proteins were transferred to nitrocellulose membranes. The membranes were incubated in PBST containing 5% skim milk for 1 h to block nonspecific protein binding. The proteins were then immunoblotted with specific antibodies (1:1000 dilution) overnight at 4 °C. The membranes were incubated with an HRP-conjugated secondary antibody (1:5000 dilution) for 1 h at room temperature and developed using an ECL chemiluminescence system. The LAS3000-mini system (Fujifilm, Tokyo, Japan) was used for detection.

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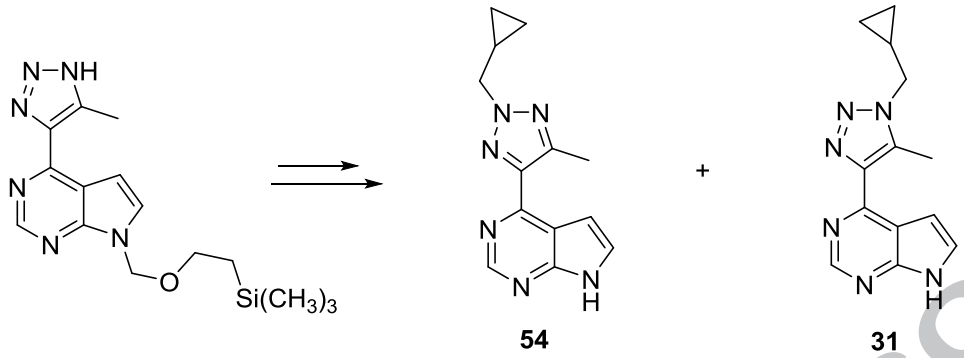
**Graphic abstract**




**54** **31**

| **54** | IC $_{50}$ (nM) |
|---|---|
| JAK1 | 444 |
| JAK2 | 41.9 |
| JAK3 | 2400 |

## Highlights

1. We discovered a potent and selective inhibitor of JAK2, compared to JAK1 and -3.
2. 2,5-triazole regioisomers showed a tendency to inhibite JAK2 kinase activity.
3. **54** exhibited 41.9 nM of $IC_{50}$ , and fold selectivity ( JAK1/2 10.6 , JAK3/2 58.1).