Supporting Information:

Discovery of the Macrocycle 11-(2-Pyrrolidin-1-yl-ethoxy)-14,19-dioxa-5,7,26-triaza-tetracyclo[19.3.1.1(2,6).1(8,12)]heptacosa-1(25),2(26),3,5,8,10,12(27),16,21,23-decaene (SB1518), a Potent Janus Kinase 2/Fms-LikeTyrosine Kinase-3 (JAK2/FLT3) Inhibitor for the Treatment of Myelofibrosis and Lymphoma

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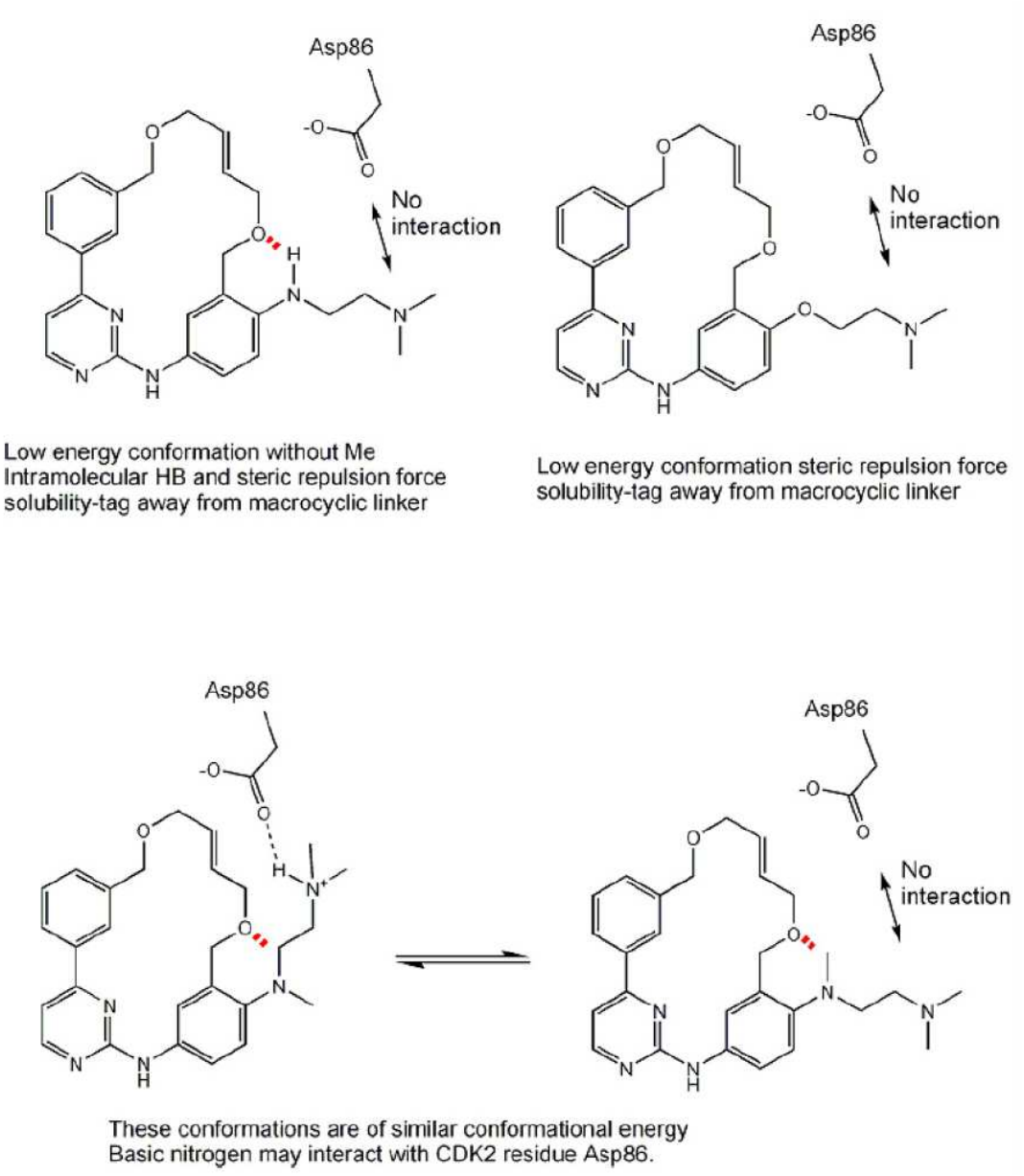
Contents of SI:

Explanation for CDK2 potency of **17h** , Western blot analyses materials and methods of **21c** and structures of RCM catalysts Grubbs 2nd generation and Zhan-1B

Figure S1: Explanation for CDK2 potency of **17h** (*N*,*N*-dimethylaminoethyl(*N*'-methyl)amino): with the *N*-methyl linker there is a negligible energy difference between the two conformations available to the side-chain (similar steric interaction with the nearby macrocycle oxygen) hence the side chain can achieve an electrostatic interaction with Asp86. In contract the oxygen or NH linkers have an energy preference to remain in a conformation which does not allow interaction between the terminal dimethylamino group and Asp86 hence giving rise to greater CDK2/JAK2 selectivity.



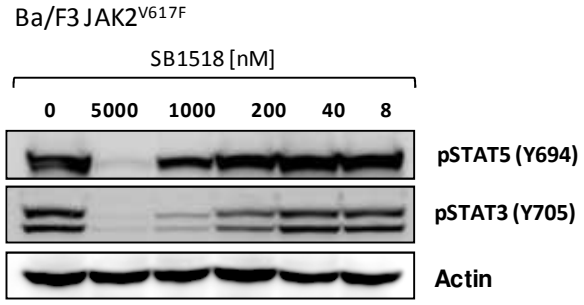


Figure S2: 21c effectively blocks signaling pathways in JAK2^{V617F} expressing cell lines. Ba/F3-JAK2^{V617F} cells were treated for 3 h with SB1518. After lysis, pJAK2 (Y1007/8) was detected by immunoblotting (IB) with anti-pSTAT3(Y705), antibody, followed by re-probing of the same membrane with anti-pSTAT5(Y694) and anti-actin antibody.

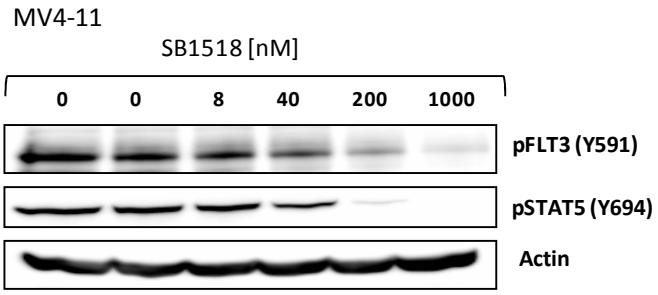


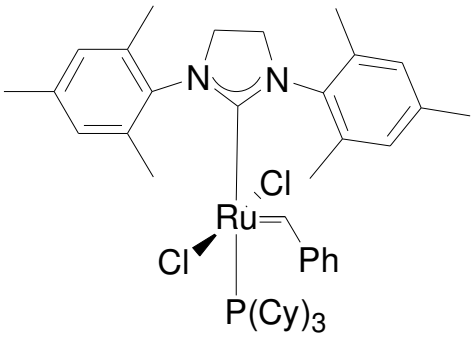
Figure S3: 21c effectively blocks FLT3 signaling in FLT3-ITD harboring cells.

MV4-11 cells were treated with SB1518 for 3 hr as indicated. After lysis, pFLT3 (Y591) was detected by immunoblotting (IB) with anti-p(Y591)FLT3 antibody, pSTAT5(Y694) was detected by IB with anti-p(Y694)STAT5 antibody. As a loading control, the same membranes were re-probed with anti-actin antibody.

Western Blot analyses Materials and Methods

Cell lysis, protein quantification and Western blots were performed as described previously (Novotny-Diermayr, V.; Sangthongpitag K.; Hu, C.Y.; Wu, X.; Sausgruber, N.; Yeo, P.; et al. SB939, a novel potent and orally active histone deacetylase inhibitor with high tumor exposure and efficacy in mouse models of colorectal cancer. *Mol Cancer Ther* **2010**, *9*, 642-652). Following SDS-polyacrylamide gel electrophoresis, proteins were transferred to PVDF membranes. Western blots were performed according to standard methods. pFLT3 (Y591) (Cat #3461), pSTAT3 (Y705), anti-mouse IgG (Cat #7074), and anti-rabbit IgG, HRP-linked (Cat #7076) antibodies were purchased from Cell Signaling Technology (Beverly, MA). pSTAT5 (Y694, Cat #611965) was obtained from BD Biosciences (San Jose, CA) and actin (Cat #2066) from Sigma (St Louis, MO). After the final wash, membranes were incubated for 5 min in ECL from GE-Healthcare (Singapore). The images were captured digitally using the LAS-3000 Life Science Imager from Fujifilm (Tokyo, Japan) using exposures between 200 s and 2000 s at normal sensitivity. Densitometric analysis was performed using the MultiGauge software (v3.1) from Fujifilm.

Grubbs 2nd generation catalyst ($C_{46}H_{65}Cl_2N_2PRu$): [1,3-Bis(2,4,6-trimethylphenyl)-2 imidazolidinylidene]dichloro(phenylmethylene)(tricyclohexylphosphine)ruthenium:



Zhan-1B catalyst ($RuCl_2[C_{21}H_{26}N_2][C_{12}H_{17}NO_3S]$): 1,3-Bis(2,4,6-trimethylphenyl)-4,5-dihydroimidazol-2-ylidene[2-(i-propoxy)-5-(N,N-dimethylaminosulfonyl)phenyl]methyleneruthenium (II) dichloride:

http://www.zannanpharma.com/en/product.asp