Supporting Information

Discovery of JAK2/3 inhibitors from quinoxalinone-containing compounds

Kamonpan Sanachai[1], Panupong Mahalapbutr[2], Lueacha Tabtimmai[3], Supaphorn Seetaha[4], Tanakorn Kittikool[5], Sirilata Yotphan[5], Kiattawee Choowongkomon[4,*], Thanyada Rungrotmongkol[1,6,*]

[1] Center of Excellence in Structural and Computational Biology Research Unit, Department of Biochemistry, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand

[2] Department of Biochemistry, and Center for Translational Medicine, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand

[3] Department of Biotechnology, Faculty of Applied Science, King Mongkut's University of Technology of North Bangkok, Bangkok 10800, Thailand

[4] Department of Biochemistry, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand

[5] Department of Chemistry and Center of Excellence for Innovation in Chemistry, Faculty of Science, Mahidol University, Rama VI Road, Bangkok 10400, Thailand

[6] Program in Bioinformatics and Computational Biology, Graduate School, Chulalongkorn University, Bangkok 10330, Thailand

[*]Corresponding authors. Tel: +66 2 2185426 and +66 2 5625444

E-mail address: t.rungrotmongkol@gmail.com and fsciktc@ku.ac.th

Contents

I. Supplemental Figures

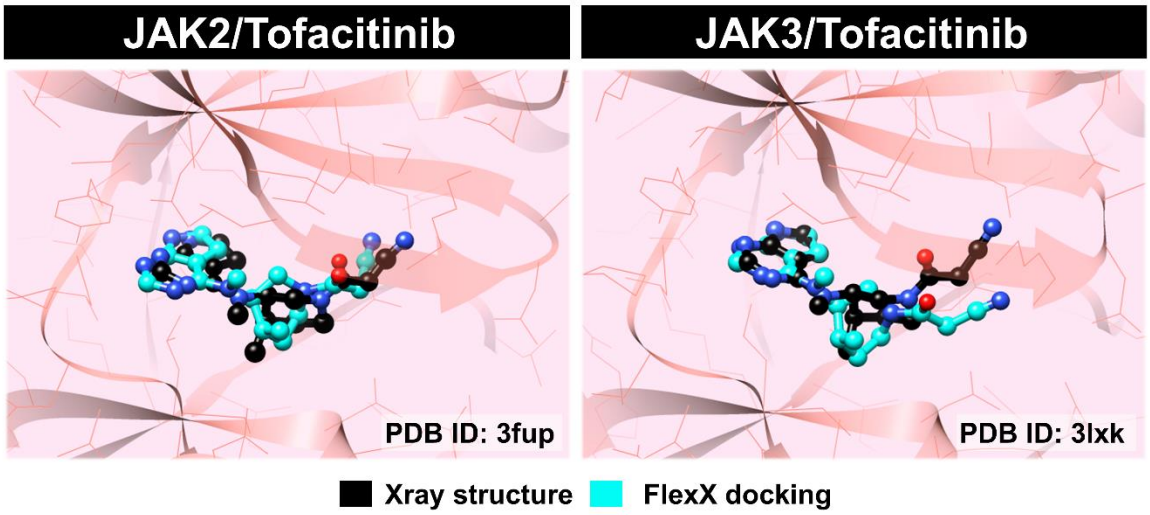

Figure S1. Superimposition of tofacitinib towards JAK2/3 between x-ray structure and FlexX docking generation.

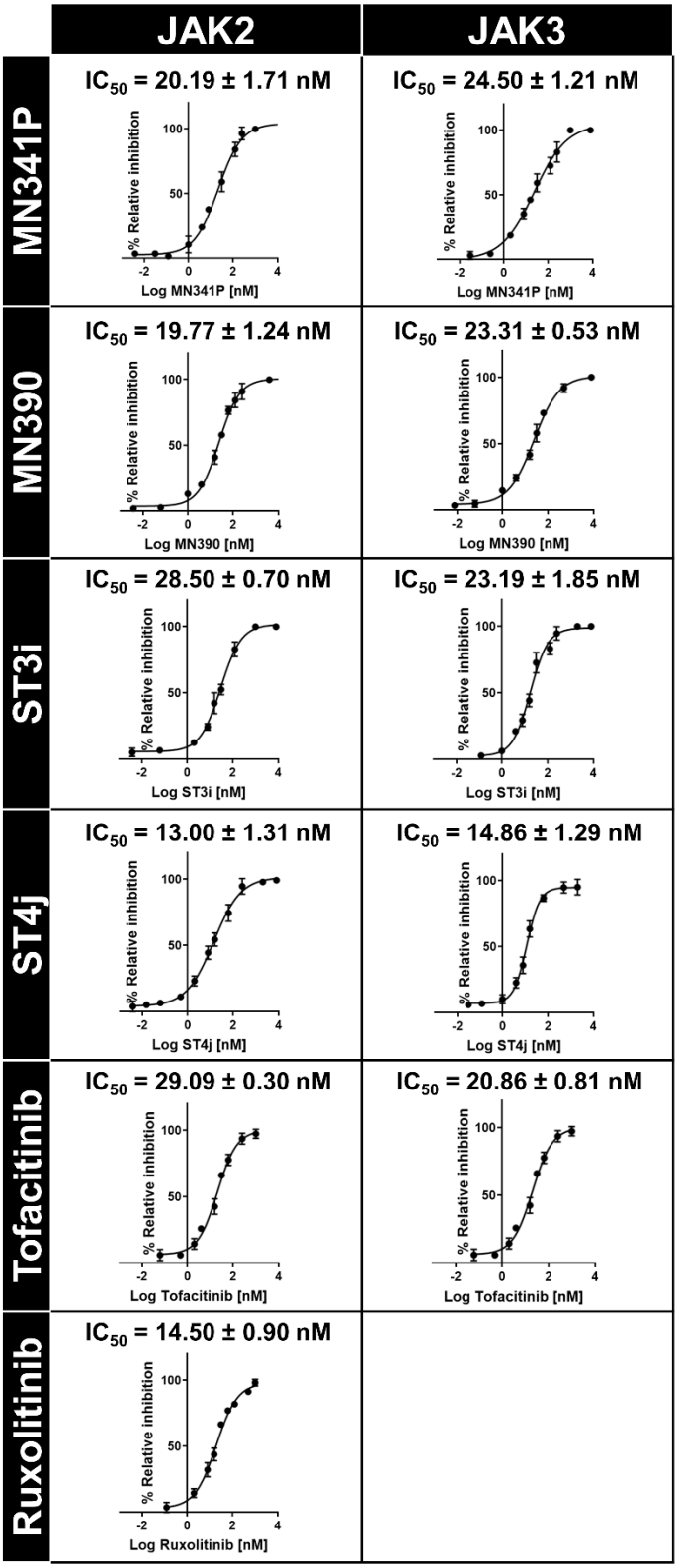

Figure S2. The IC_{50} curve of potent compounds (MN341P, MN390, ST3i and ST4j) and drugs (tofacitinib and ruxolitinib) towards JAK2/3.

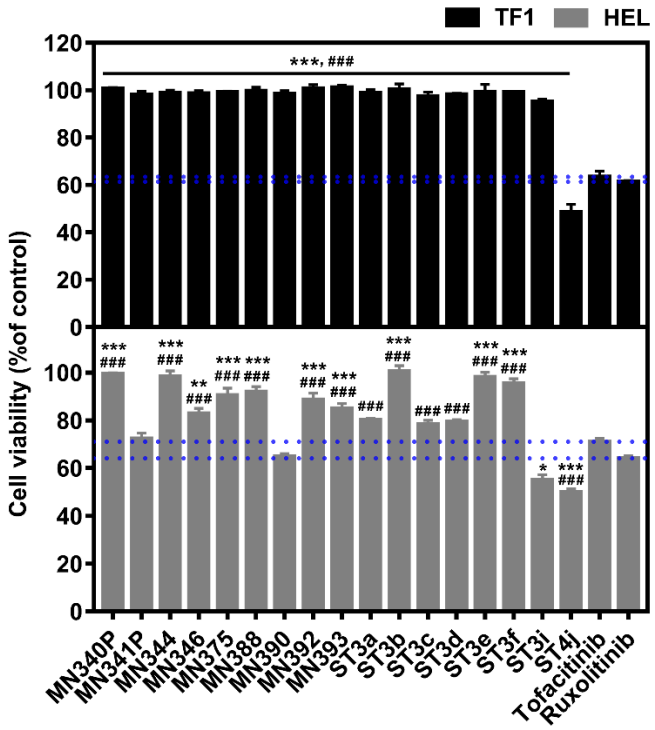

Figure S3. Cellular cytotoxicity assay of TF1 and HEL cells treated with quinoxalinone derivatives at 10 μM concentrations. * $p \leq 0.05$, ** $p \leq 0.01$ and *** $p \leq 0.001$ vs. tofacitinib and $^{###}$ $p \leq 0.001$ vs. ruxolitinib.

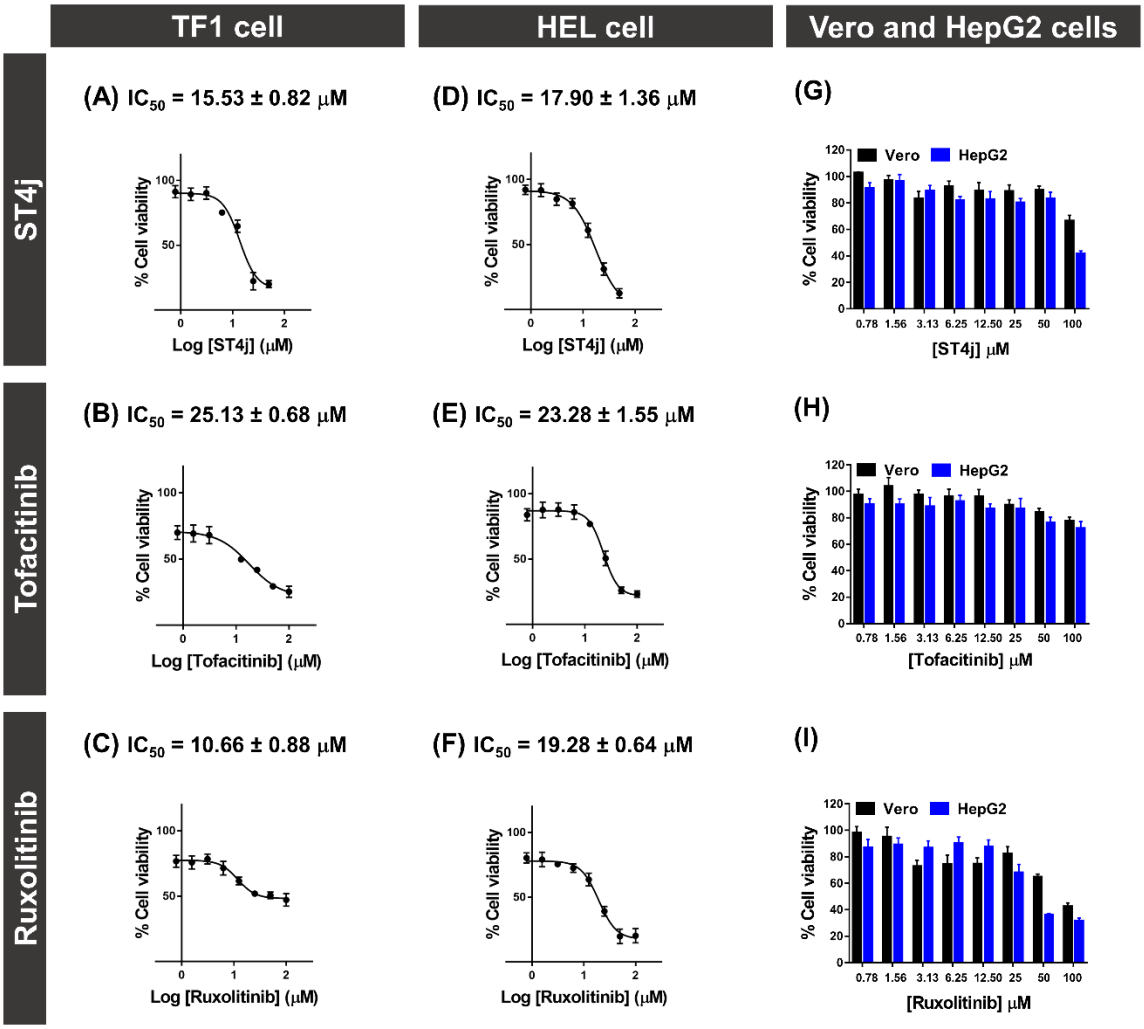

Figure S4. Cell viability after treatment for 72 h of cell lines: (A-C) TF1 cell line, (D-F) HEL cell line and (G-I) Vero and HepG2 cell lines with ST4j and drugs (tofacitinib and ruxolitinib). The IC_{50} values are reported in μM from triplicate independent experiments.

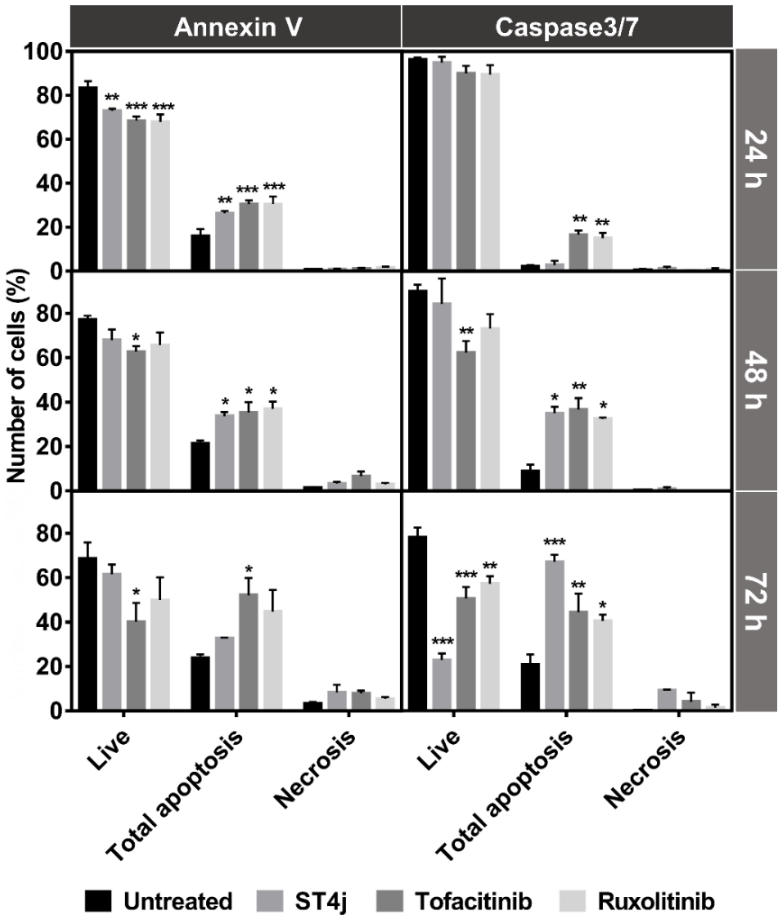

Figure S5. Flow cytometry analysis of time-dependent induced-apoptotic TF1 cells treated with IC_{50} values of ST4j compound and drugs (tofacitinib and ruxolitinib) for 24 h, 48h and 72 h, respectively. Bar chart showing an increased proportion of apoptotic cells derived from annexin V and caspase3/7. Data are represented as mean ± SEM derived from the triplicate independent experiment.

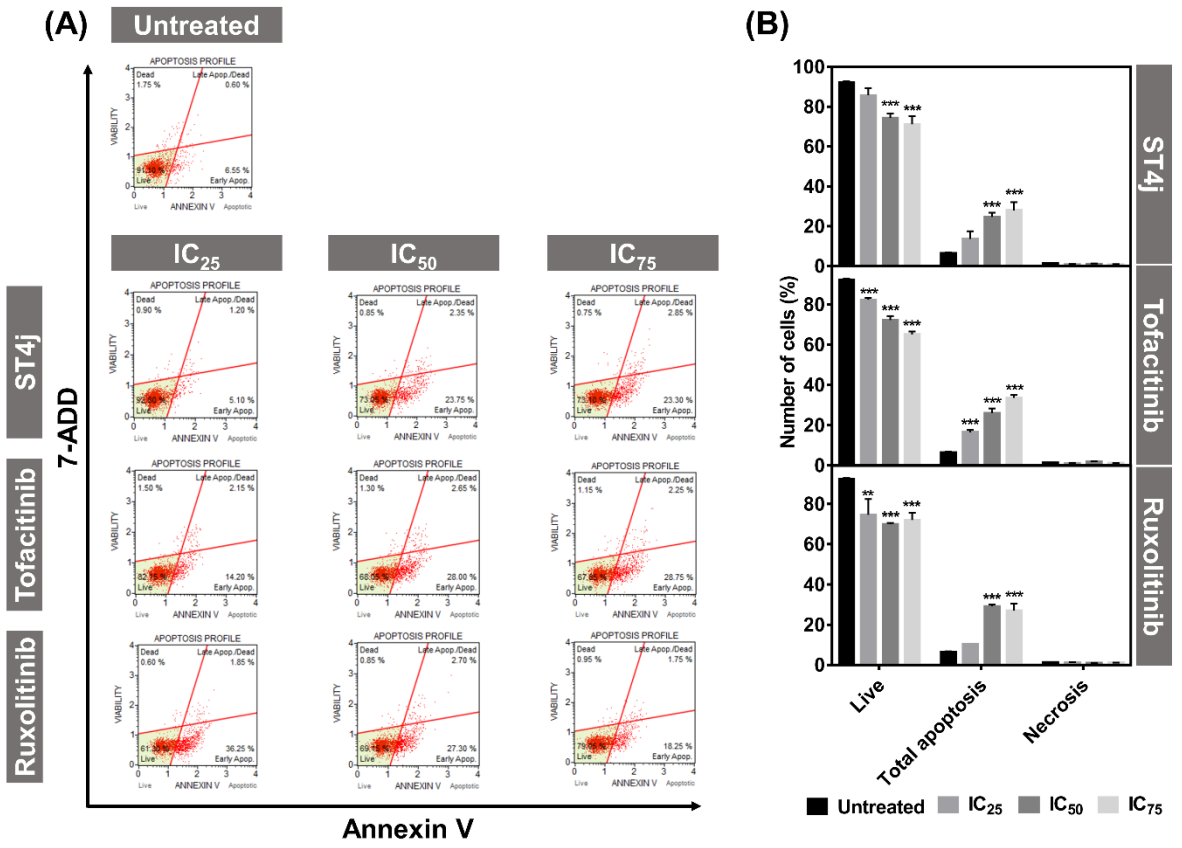

Figure S6. Flow cytometry analysis from annexin V of dose-dependent induced-apoptotic TF1 cells treated with various concentrations (IC_{25}, IC_{50} and IC_{75} values) of the ST4j compound and drugs (tofacitinib and ruxolitinib) for 24 h. (A) representative figures showing the population of live, apoptosis and dead cells, and (B) bar chart showing an increased proportion of apoptotic cells. Data are represented as mean ± SEM derived from the triplicate independent experiment.

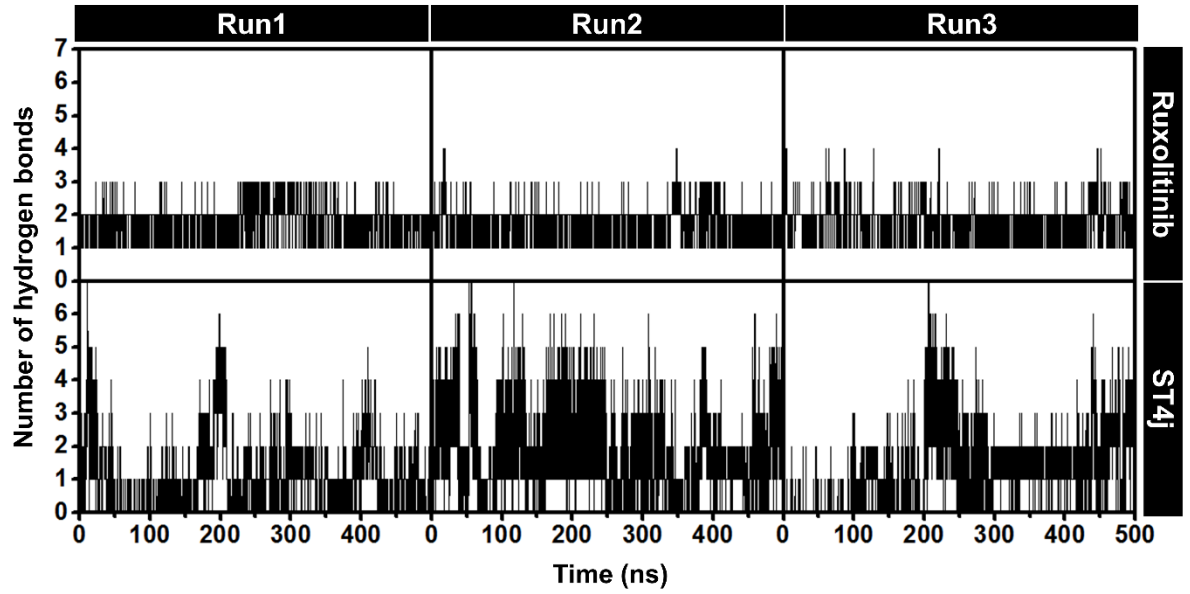

Figure S7. The number of hydrogen bonds formed between JAK2 and ruxolitinib/ST4j along 500 ns of simulation. The hydrogen bond occupations of complexes were observed as follows: (i) ≤3.5 Å for distance and (ii) ≥120° for the angle.

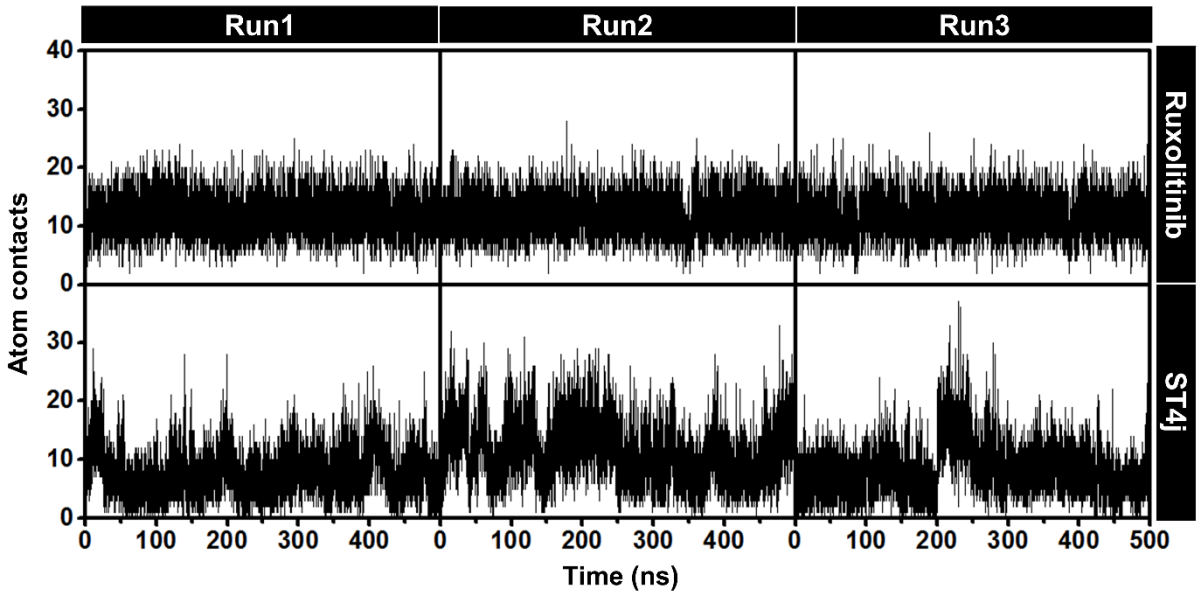

Figure S8. The number of atom contacts between JAK2 and ruxolitinib/ST4j along 500 ns of simulation.