

## Supporting information

### **RSK1 vs RSK2 inhibitory activity of the marine $\beta$ -carboline alkaloid Manzamine A: a biochemical, cervical cancer protein expression and computational docking study**

**Alejandro M.S. Mayer<sup>1\*</sup>, Mary L. Hall<sup>1</sup>, Joseph Lach<sup>1</sup>, Jonathan Clifford<sup>1</sup>, Kevin Chandrasena<sup>1</sup>, Caitlin Canton<sup>1</sup>, Maria Kontoyianni<sup>2</sup>, Yeun-Mun Choo<sup>3</sup>, Dev Karan<sup>4</sup>, and Mark T. Hamann<sup>5</sup>**

<sup>1</sup> Department of Pharmacology, College of Graduate Studies, Midwestern University, 555 31<sup>st</sup> Street, Downers Grove, Illinois 60515, USA; E-Mails: mhall1@midwestern.edu (M.L.H.); joseph.m.lach@gmail.com (J.L.); clifford07@gmail.com (J.C.); kschandras@gmail.com (K.C.); canto030@umn.edu (C.C.)

<sup>2</sup> Department of Pharmaceutical Sciences, Southern Illinois University Edwardsville, Edwardsville, Illinois 62026-2000, USA; E-Mail: mkontoy@siue.edu

<sup>3</sup> Department of Chemistry, University of Malaya, Kuala Lumpur 50603, Malaysia; E-Mail: ymchoo@um.edu.my

<sup>4</sup> Laboratory Medicine, Department of Pathology, Medical College of Wisconsin, Milwaukee, WI; E-Mail: dkaran@mcw.edu

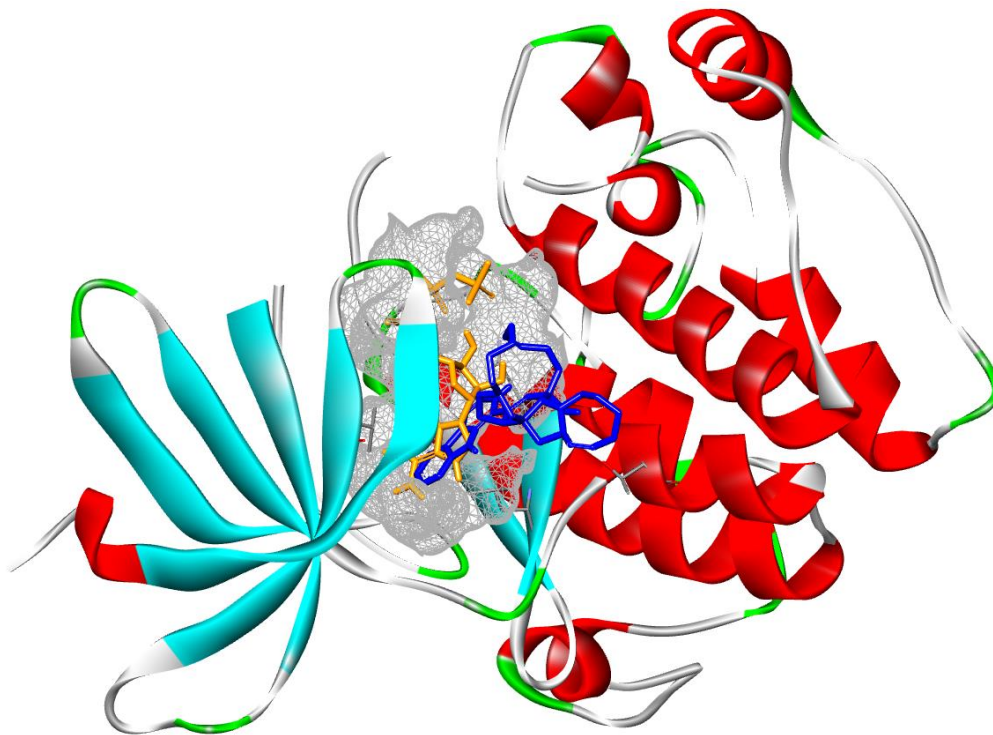
<sup>5</sup> Department of Drug Discovery and Biomedical Sciences and Public Health, Colleges of Pharmacy and Medicine, Medical University of South Carolina, Charleston, South Carolina. 29425, USA; E-Mail: hamannm@musc.edu

\* Author to whom correspondence should be addressed; E-Mail: amayer@midwestern.edu  
Tel.: (630) 515-6951; Fax: (630) 515-6295.

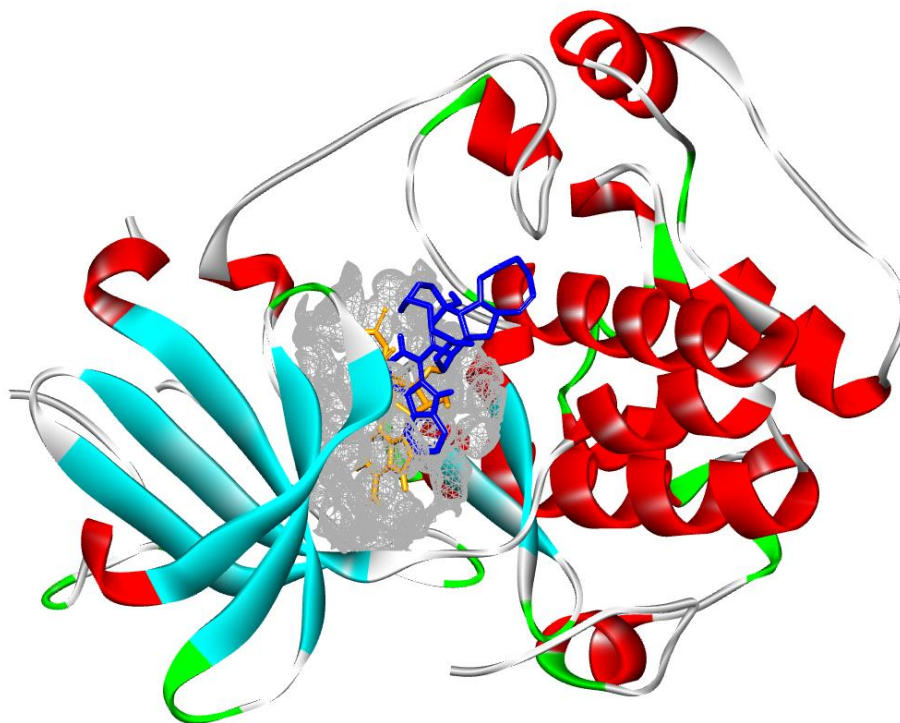
## List of figures

- 1) **Figure S1:** MZA (blue) and ATP (orange) is shown docked to MAPK-activated protein kinase-1 $\alpha$  (binding pocket is shown as gray mesh) (MAPKAP-K1 $\alpha$ /RSK1) (PDB ID: 2Z7Q)
- 2) **Figure S2:** MZA (blue) and ATP (orange) is shown docked to serum and glucocorticoid-induced kinase (binding pocket is shown as gray mesh) (SGK) (PDB ID: 2R5T)
- 3) **Figure S3:** MZA (blue) and ATP (orange) is shown docked to lymphocyte kinase (binding pocket is shown as gray mesh) (LCK) (PDB ID: 3MPM)
- 4) **Figure S4:** MZA (blue) and ATP (orange) is shown docked to mitogen and stress-activated protein kinase-1 (binding pocket is shown as gray mesh) (MSK1) (PDB ID: 3KN5)
- 5) **Figure S5:** MZA (blue) and ATP (orange) is shown docked to glycogen synthase kinase-3 $\beta$  (binding pocket is shown as gray mesh) (GSK3 $\beta$ ) (PDB ID: 1Q5K)
- 6) **Figure S6:** MZA (blue) and ATP (orange) is shown docked to stress-activated protein kinase-2 $\alpha$  (binding pocket is shown as gray mesh) (SAPK2 $\alpha$ /P38) (PDB ID: 5OMH)
- 7) **Figure S7:** MZA (blue) and ATP (orange) is shown docked to protein kinase B (binding pocket is shown as gray mesh) (PKB $\alpha$ ) (PDB ID: 3CQW)
- 8) **Figure S8:** MZA (blue) and ATP (orange) is shown docked to c-Jun N-terminal kinase (a binding pocket is shown as gray mesh) (JNK1 $\alpha$ 1) (PDB ID: 1UKI)
- 9) **Figure S9:** MZA (blue) and ATP (orange) is shown docked to p70 ribosomal protein S6 kinase (binding pocket is shown as gray mesh) (S6K1) (PDB ID: 3A60)
- 10) **Figure S10:** MZA (blue) and ATP (orange) is shown docked to dual tyrosine phosphorylated and regulated kinase 1A (binding pocket is shown as gray mesh) (DYRK1A) (PDB ID: 2VX3)
- 11) **Figure S11:** MZA (blue) and ATP (orange) is shown docked to casein kinase-2 (binding pocket is shown as gray mesh) (CK2) (PDB ID: 1J91)
- 12) **Figure S12:** MZA (blue) and ATP (orange) is shown docked to checkpoint kinase-1 (binding pocket is shown as gray mesh) (CHK1) (PDB ID: 3U9N)
- 13) **Figure S13:** MZA (blue) and ATP (orange) is shown docked to C-terminal Src kinase (binding pocket is shown as gray mesh) (CSK) (PDB ID: 1BYG)
- 14) **Figure S14:** MZA (blue) and ATP (orange) is shown docked to MAPK kinase (mitogen-activated protein kinase) (binding pocket is shown as gray mesh) (MKK1) (PDB ID: 1S9J)
- 15) **Figure S15:** MZA (blue) and ATP (orange) is shown docked to cyclic AMP-dependent protein kinase (binding pocket is shown as gray mesh) (PKA) (PDB ID: 2JDS)
- 16) **Figure S16:** MZA (blue) and ATP (orange) is shown docked to protein kinase C (binding pocket is shown as gray mesh) (PKC $\alpha$ ) (PDB ID: 3IW4)
- 17) **Figure S17:** MZA (blue) and ATP (orange) is shown docked to casein kinase-1 (binding pocket is shown as gray mesh) (CK1) (PDB ID: 1CSN)
- 18) **Figure S18:** MZA (blue) and ATP (orange) is shown docked to MAPK-activated protein kinase-2 (binding pocket is shown as gray mesh) (MAPKAP-K2 (RSK-2)) (PDB ID: 3G51)

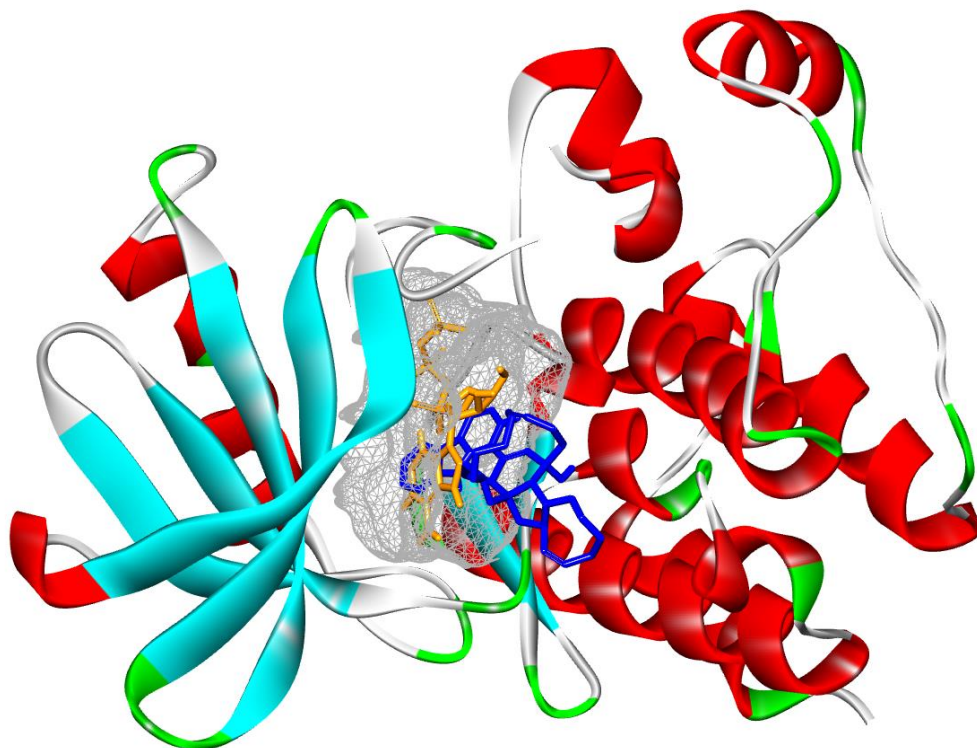
- 19) **Figure S19:** MZA (blue) and ATP (orange) is shown docked to mitogen-activated protein kinase (binding pocket is shown as gray mesh) (MAPK2/ERK2) (PDB ID: 2OJJ)
- 20) **Figure S20:** MZA (blue) and ATP (orange) is shown docked to NIMA-related protein kinase 2 $\alpha$  (binding pocket is shown as gray mesh) (NEK2 $\alpha$ ) (PDB ID: 5M53)
- 21) **Figure S21:** MZA (blue) and ATP (orange) is shown docked to AMP-activated protein kinase (binding pocket is shown as gray mesh) (AMPK) (PDB ID: 2YA3)
- 22) **Figure S22:** MZA (blue) and ATP (orange) is shown docked to stress-activated protein kinase-4 (binding pocket is shown as gray mesh) (SAPK4/P38 $\delta$ ) (PDB ID: 5EKN)
- 23) **Figure S23:** MZA (blue) and ATP (orange) is shown docked to phosphorylase kinase (binding pocket is shown as gray mesh) (PHK) (PDB ID: 2Y7J)
- 24) **Figure S24:** MZA (blue) and ATP (orange) is shown docked to stress-activated protein kinase-3 (binding pocket is shown as gray mesh) (SAPK3/P38 $\gamma$ ) (PDB ID: 1CM8)
- 25) **Figure S25:** MZA (blue) and ATP (orange) is shown docked to 3-phosphoinositide-dependent protein kinase-1 (binding pocket is shown as gray mesh) (PDK1) (PDB ID: 5LVL)
- 26) **Figure S26:** MZA (blue) and ATP (orange) is shown docked to Rho-dependent protein kinase (binding pocket is shown as gray mesh) (ROCK-II) (PDB ID: 6ED6)
- 27) **Figure S27:** MZA (blue) and ATP (orange) is shown docked to cyclin-dependent kinase 2-cyclin A complex (binding pocket is shown as gray mesh) (CDK2/cyclin A) (PDB ID: 4ez7)
- 28) **Figure S28.**  $^1\text{H}$  NMR of Manzamine A (CDCL<sub>3</sub>, 600 MHz)
- 29) **Figure S29.**  $^{13}\text{C}$  NMR of Manzamine A (CDCL<sub>3</sub>, 150 MHz)
- 30) **Figure S30.** 2D-COSY Spectrum Manzamine A (CDCL<sub>3</sub>, 600 MHz)
- 31) **Figure S31.** 2D edited g-HSQC Spectrum of Manzamine A (CDCL<sub>3</sub>, 600 MHz)



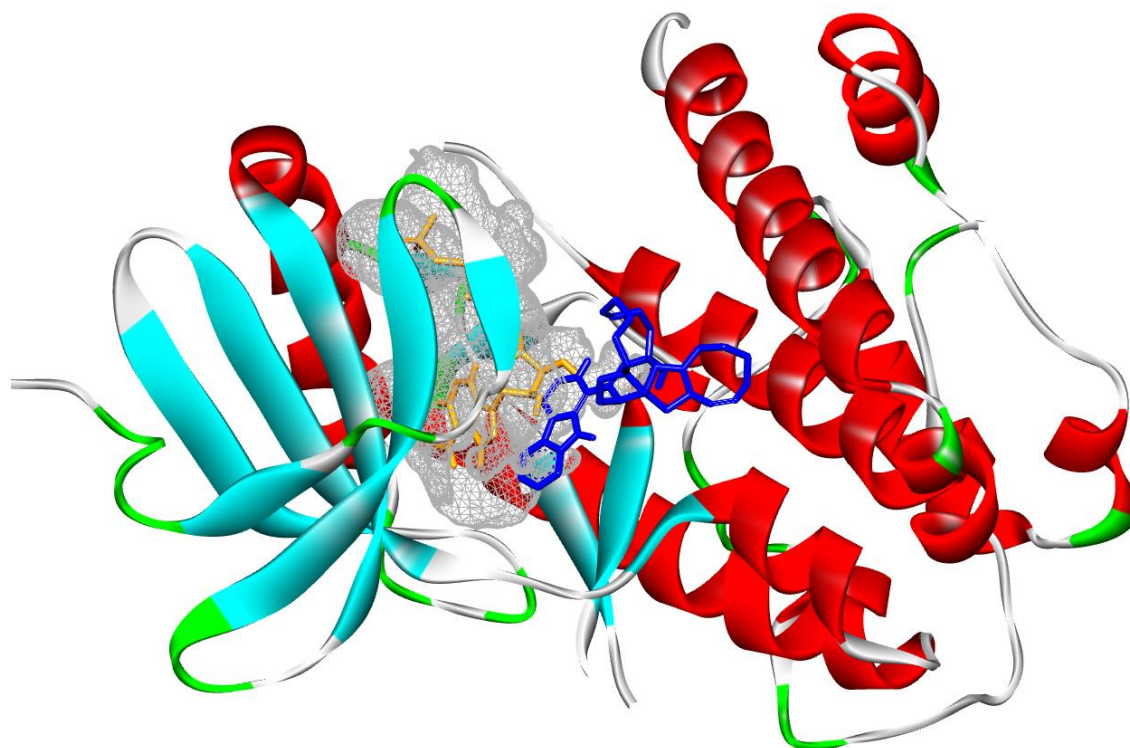
**Figure S1:** MZA (blue) and ATP (orange) is shown docked to MAPK-activated protein kinase-1 $\alpha$  (binding pocket is shown as gray mesh) (MAPKAP-K1 $\alpha$ ) (PDB ID: 2Z7Q)



**Figure S2:** MZA (blue) and ATP (orange) is shown docked to serum and glucocorticoid-induced kinase (binding pocket is shown as gray mesh) (SGK) (PDB ID: 2R5T)

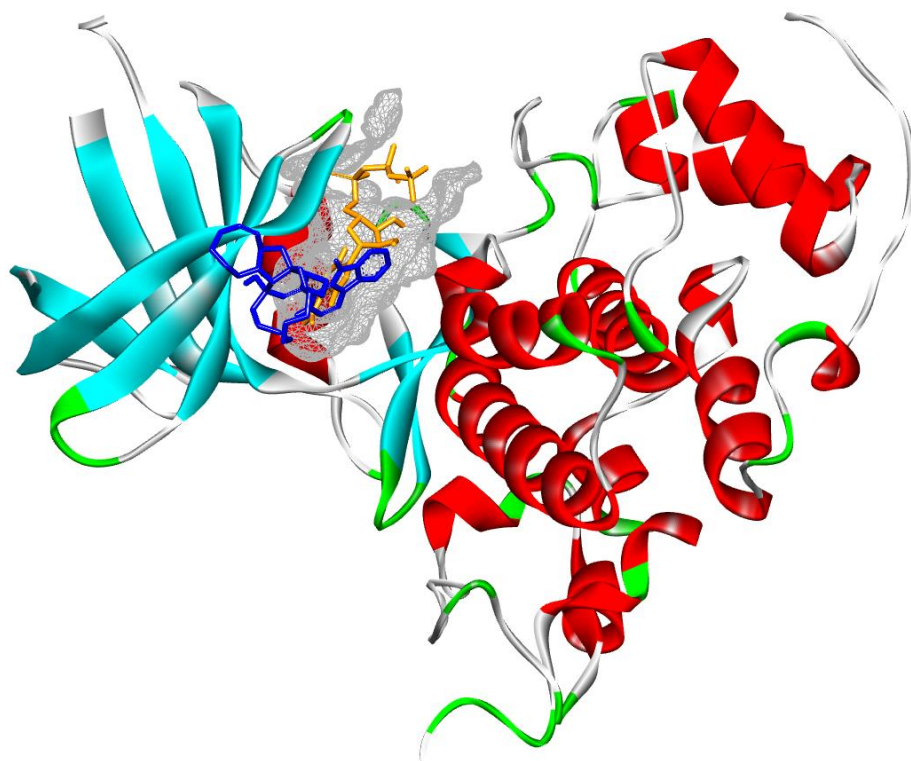


**Figure S3:** MZA (blue) and ATP (orange) is shown docked to lymphocyte kinase (binding pocket is shown as gray mesh) (LCK) (PDB ID: 3MPM)

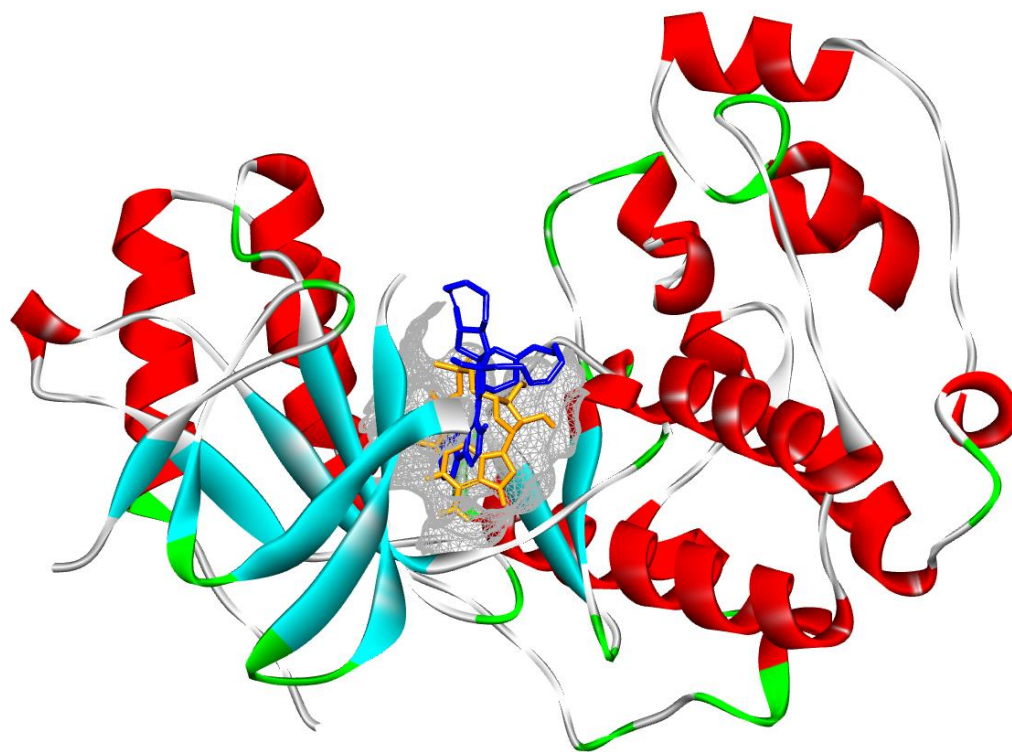


**Figure S4:** MZA (blue) and ATP (orange) is shown docked to mitogen and stress-activated protein kinase-1 (binding pocket is shown as gray mesh) (MSK1) (PDB ID: 3KN5)

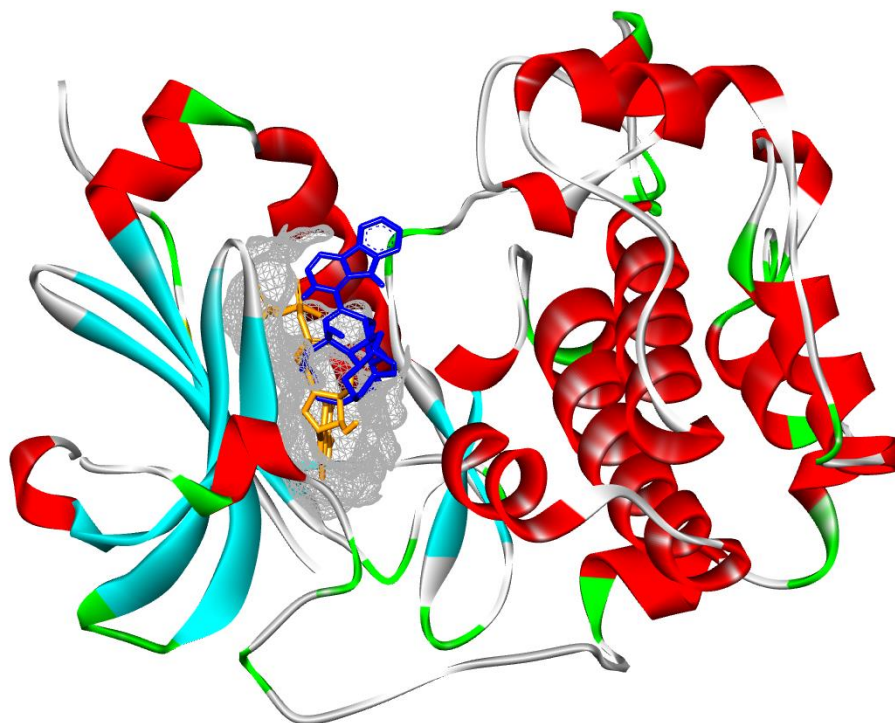




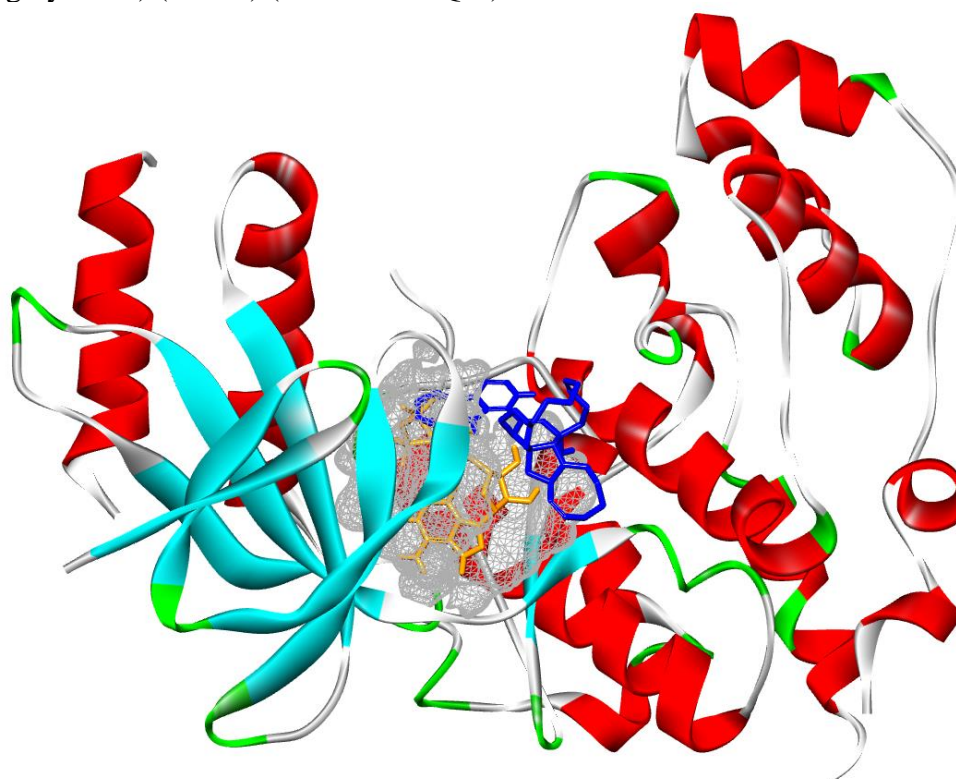
**Figure S5:** MZA (blue) and ATP (orange) is shown docked to glycogen synthase kinase-3 $\beta$  (binding pocket is shown as gray mesh) (GSK3 $\beta$ ) (PDB ID: 1Q5K)



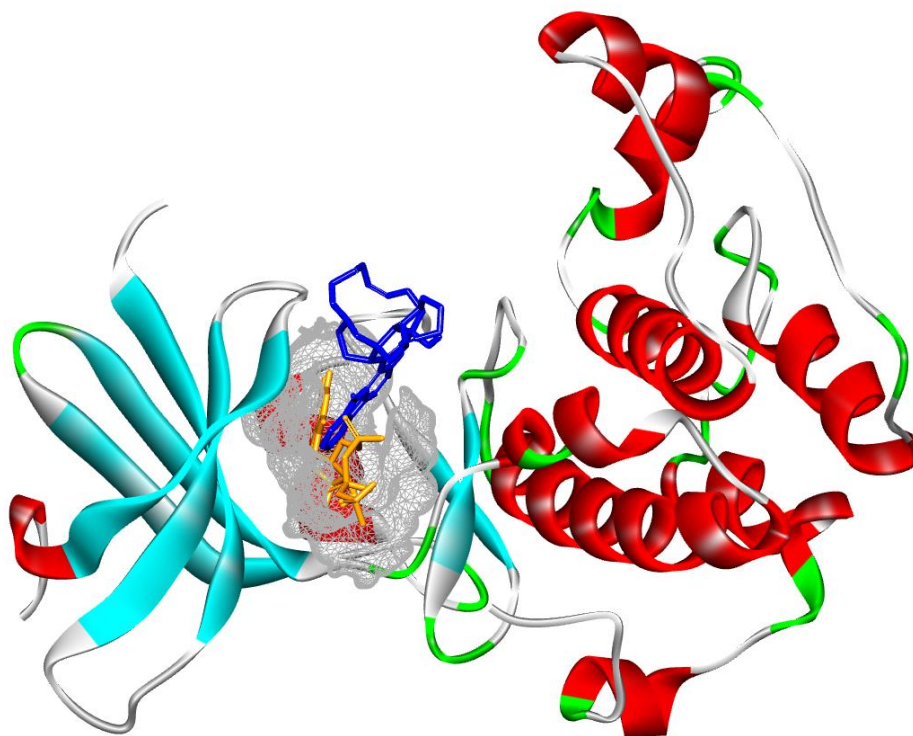
**Figure S6:** MZA (blue) and ATP (orange) is shown docked to stress-activated protein kinase-2 $\alpha$  (binding pocket is shown as gray mesh) (SAPK2 $\alpha$ /P38) (PDB ID: 5OMH)



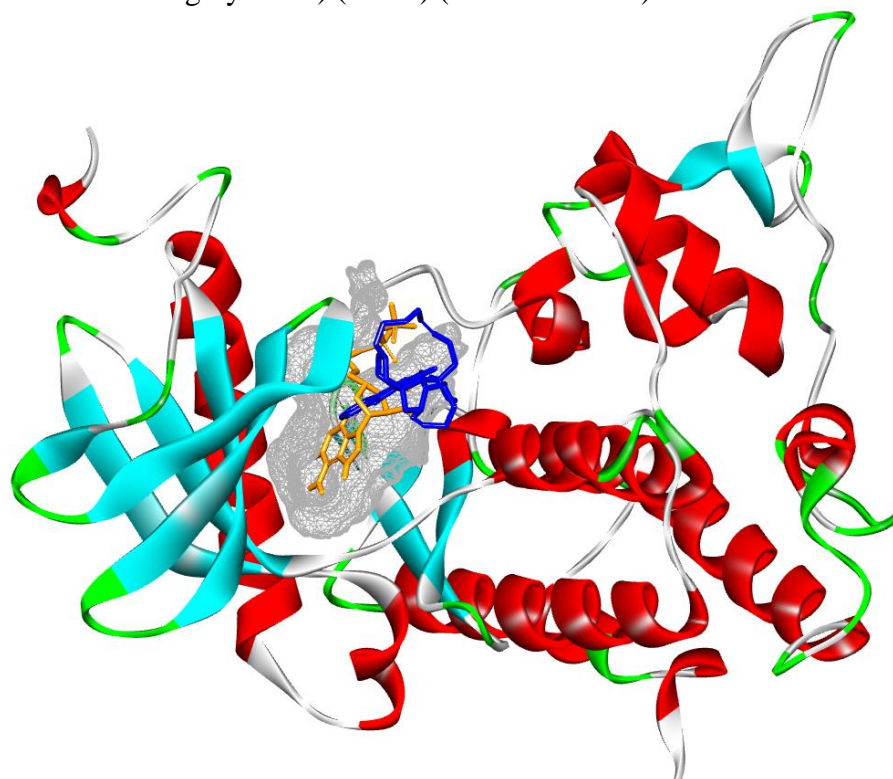
**Figure S7:** MZA (blue) and ATP (orange) is shown docked to protein kinase B (binding pocket is shown as gray mesh) (PKB $\alpha$ ) (PDB ID: 3CQW)



**Figure S8:** MZA (blue) and ATP (orange) is shown docked to c-Jun N-terminal kinase (binding pocket is shown as gray mesh) (JNK1 $\alpha$ 1) (PDB ID: 1UKI)

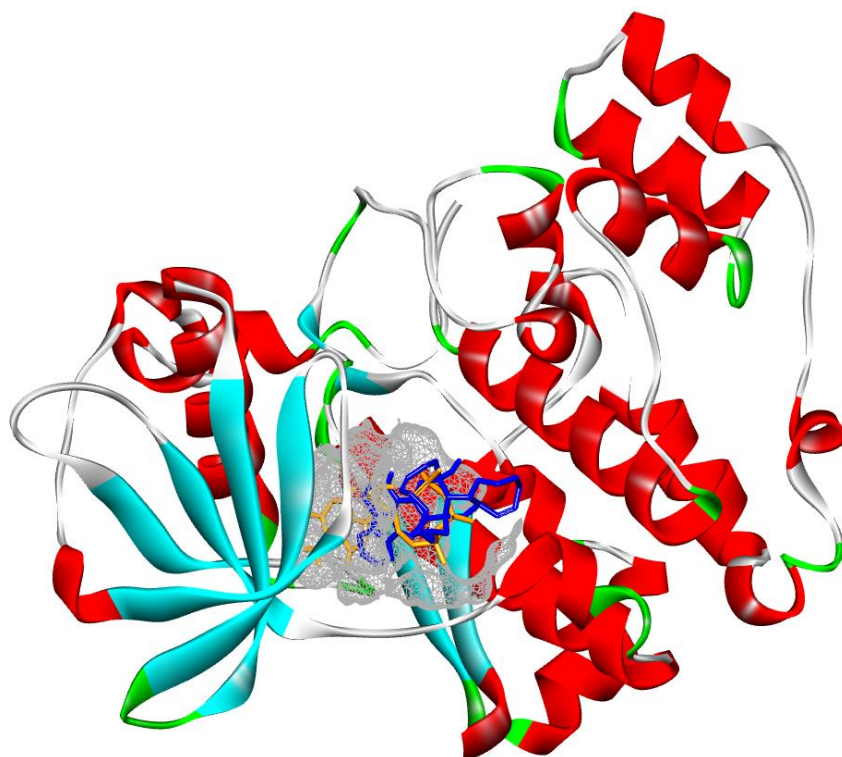


**Figure S9:** MZA (blue) and ATP (orange) is shown docked to p70 ribosomal protein S6 kinase (binding pocket is shown as gray mesh) (S6K1) (PDB ID: 3A60)

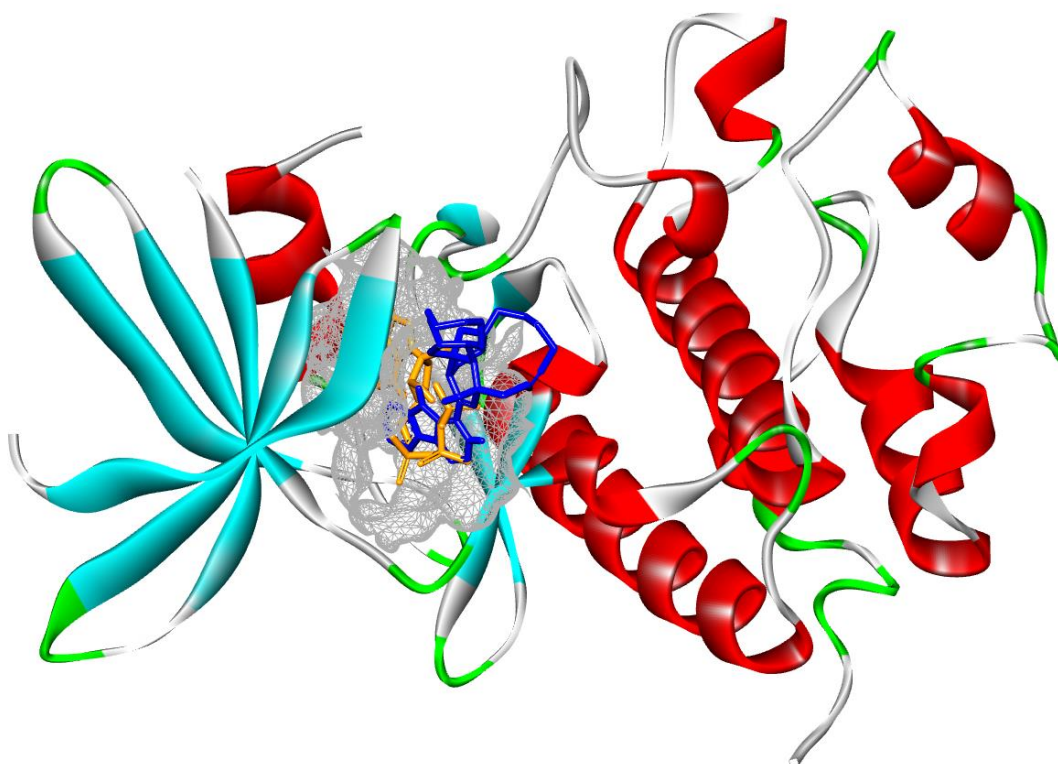


**Figure S10:** MZA (blue) and ATP (orange) is shown docked to dual tyrosine phosphorylated and regulated kinase 1A (binding pocket is shown as gray mesh) (DYRK1A) (PDB ID: 2VX3)

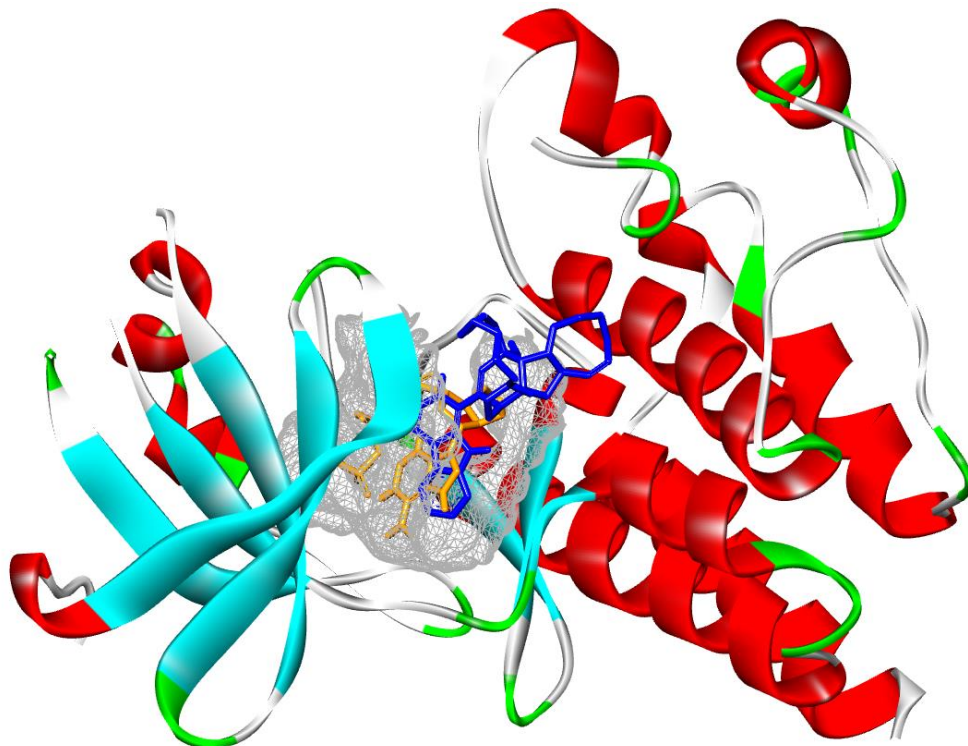




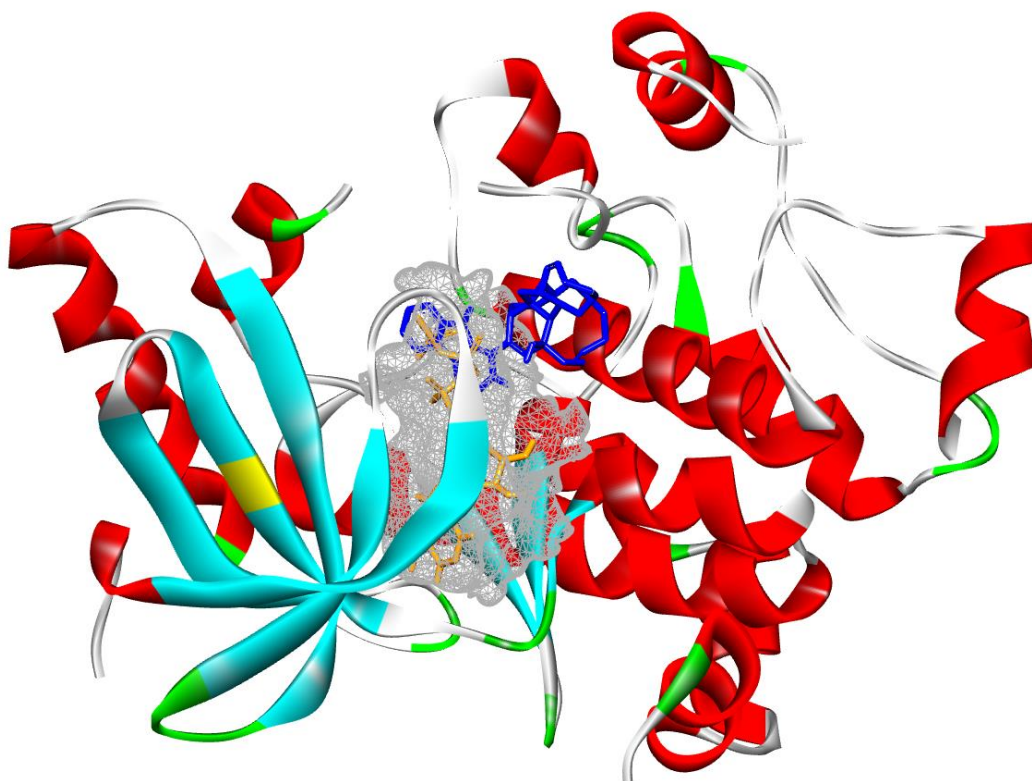
**Figure S11:** MZA (blue) and ATP (orange) is shown docked to casein kinase-2 (binding pocket is shown as gray mesh) (CK2) (PDB ID: 1J91)



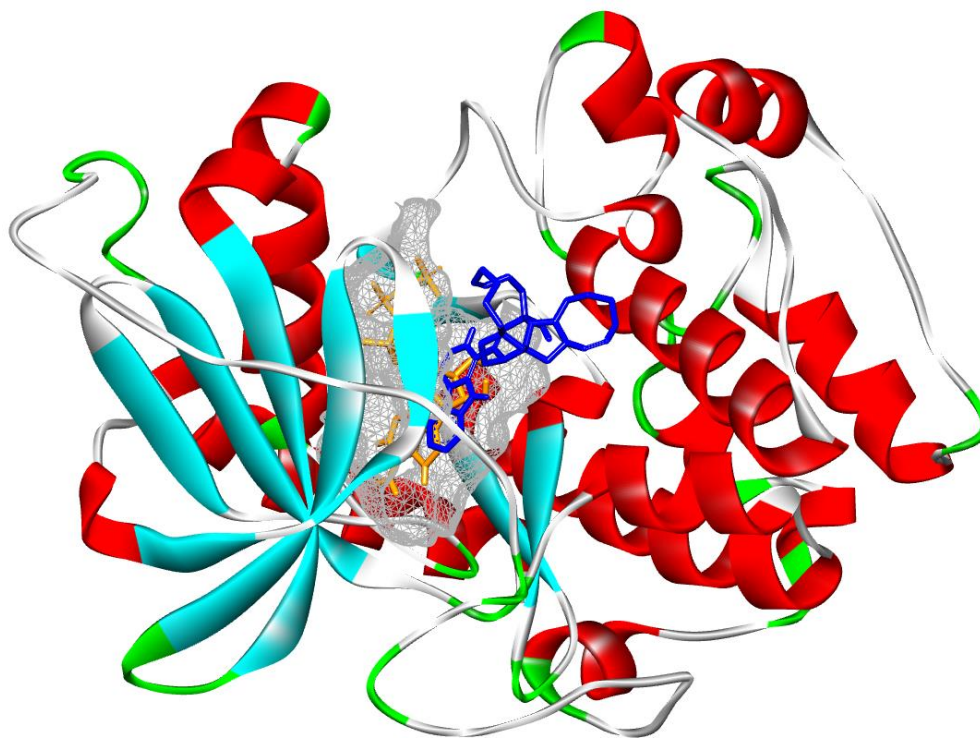
**Figure S12:** MZA (blue) and ATP (orange) is shown docked to checkpoint kinase-1 (binding pocket is shown as gray mesh) (CHK1) (PDB ID: 3U9N)



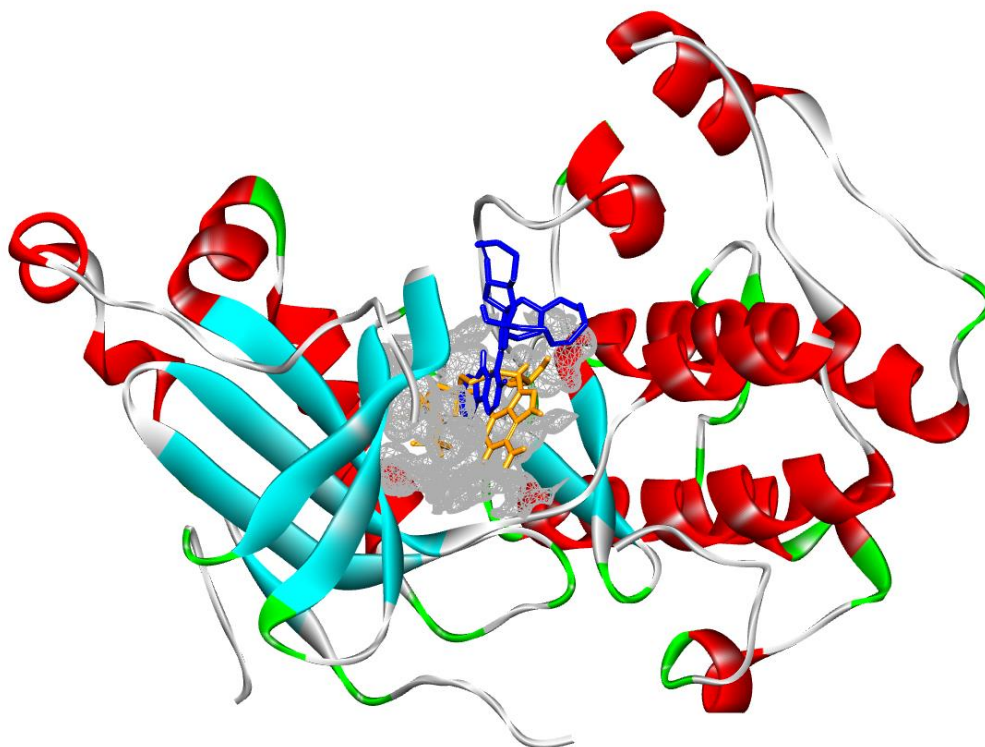
**Figure S13:** MZA (blue) and ATP (orange) is shown docked to C-terminal Src kinase (binding pocket is shown as gray mesh) (CSK) (PDB ID: 1BYG)



**Figure S14:** MZA (blue) and ATP (orange) is shown docked to MAPK kinase (mitogen-activated protein kinase) (binding pocket is shown as gray mesh) (MKK1) (PDB ID: 1S9J)

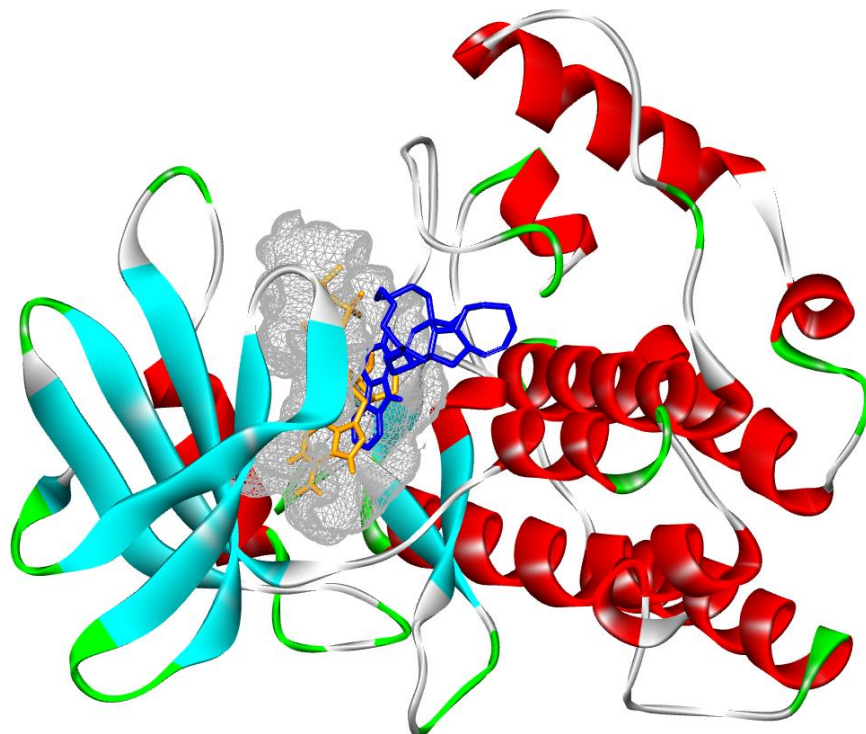


**Figure S15:** MZA (blue) and ATP (orange) is shown docked to cyclic AMP-dependent protein kinase (binding pocket is shown as gray mesh) (PKA) (PDB ID: 2JDS)

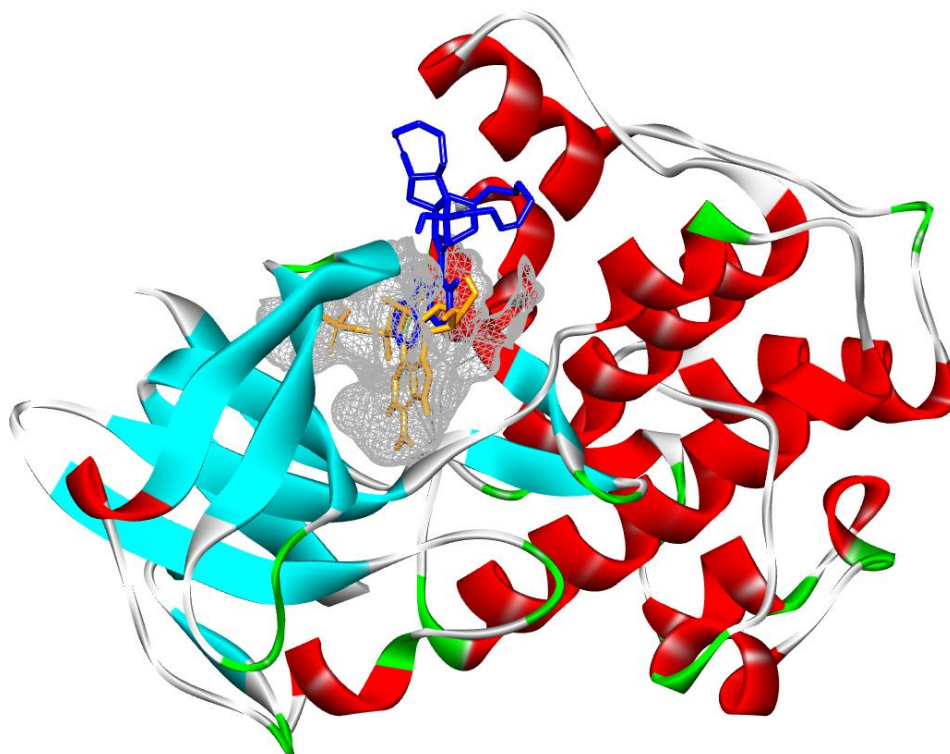


**Figure S16:** MZA (blue) and ATP (orange) is shown docked to protein kinase C (binding pocket is shown as gray mesh) (PKCα) (PDB ID: 3IW4)



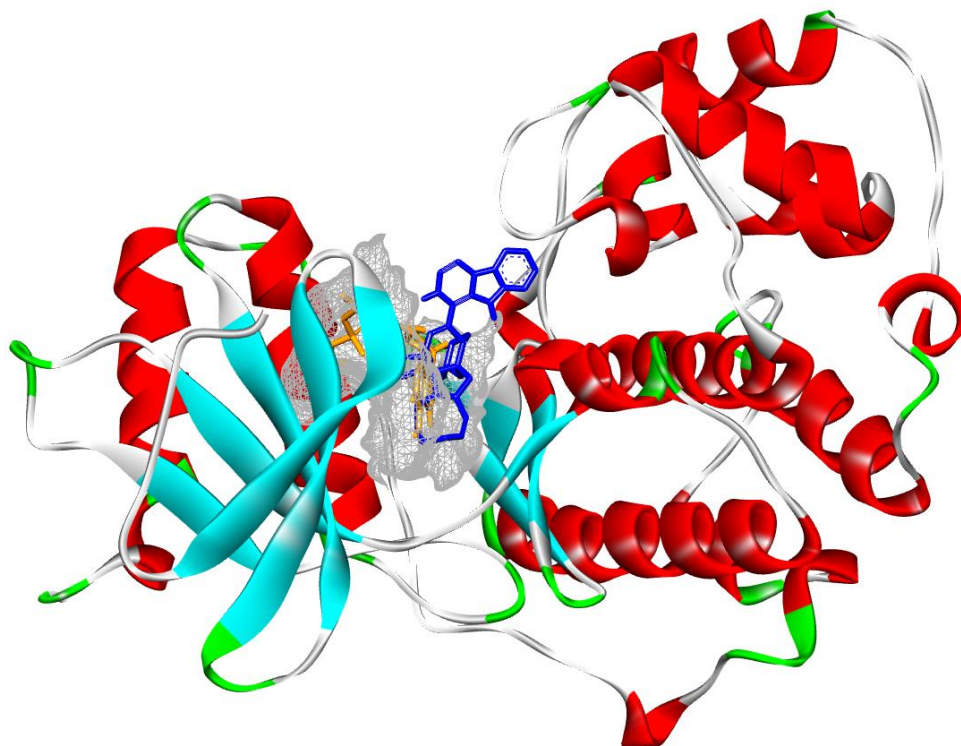


**Figure S17:** MZA (blue) and ATP (orange) is shown docked to casein kinase-1 (binding pocket is shown as gray mesh) (CK1) (PDB ID: 1CSN)

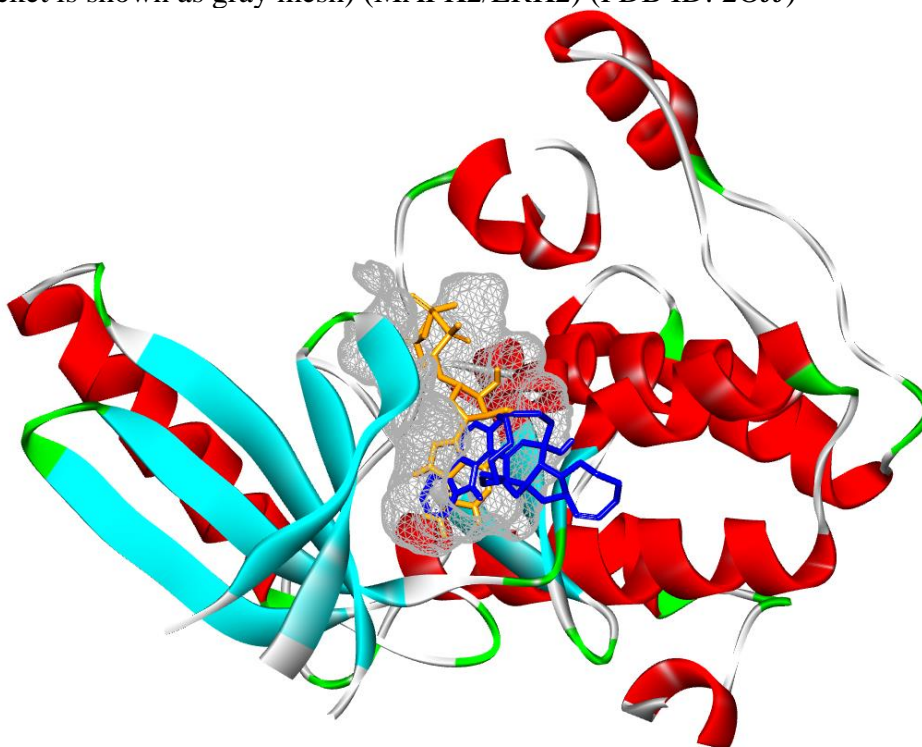


**Figure S18:** MZA (blue) and ATP (orange) is shown docked to MAPK-activated protein kinase-2 (binding pocket is shown as gray mesh) (MAPKAP-K2 (RSK-2)) (PDB ID: 3G51)

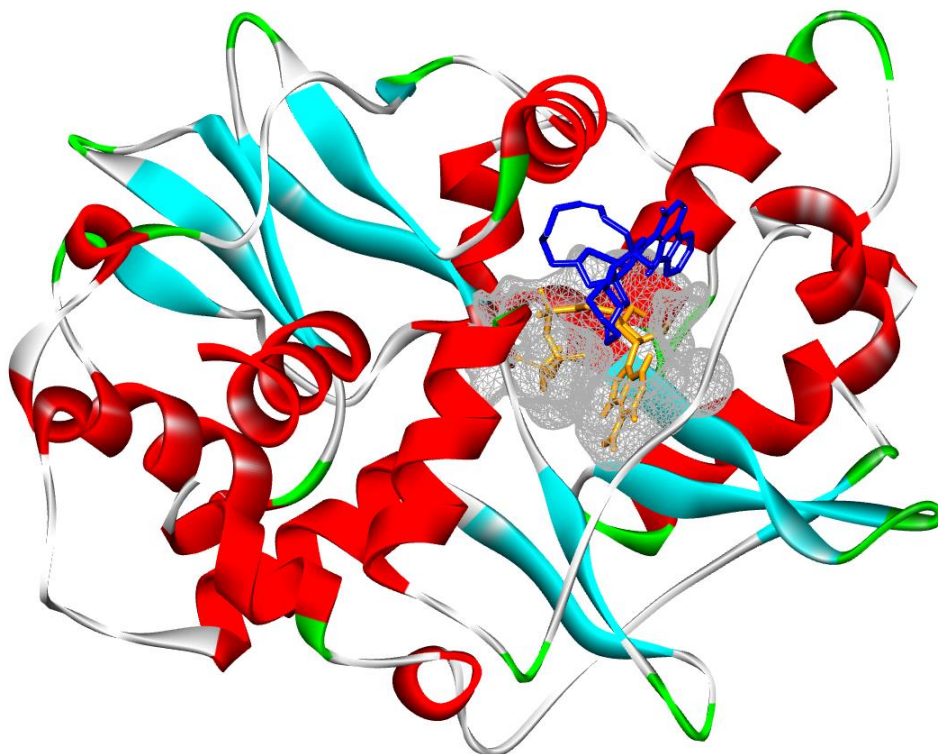




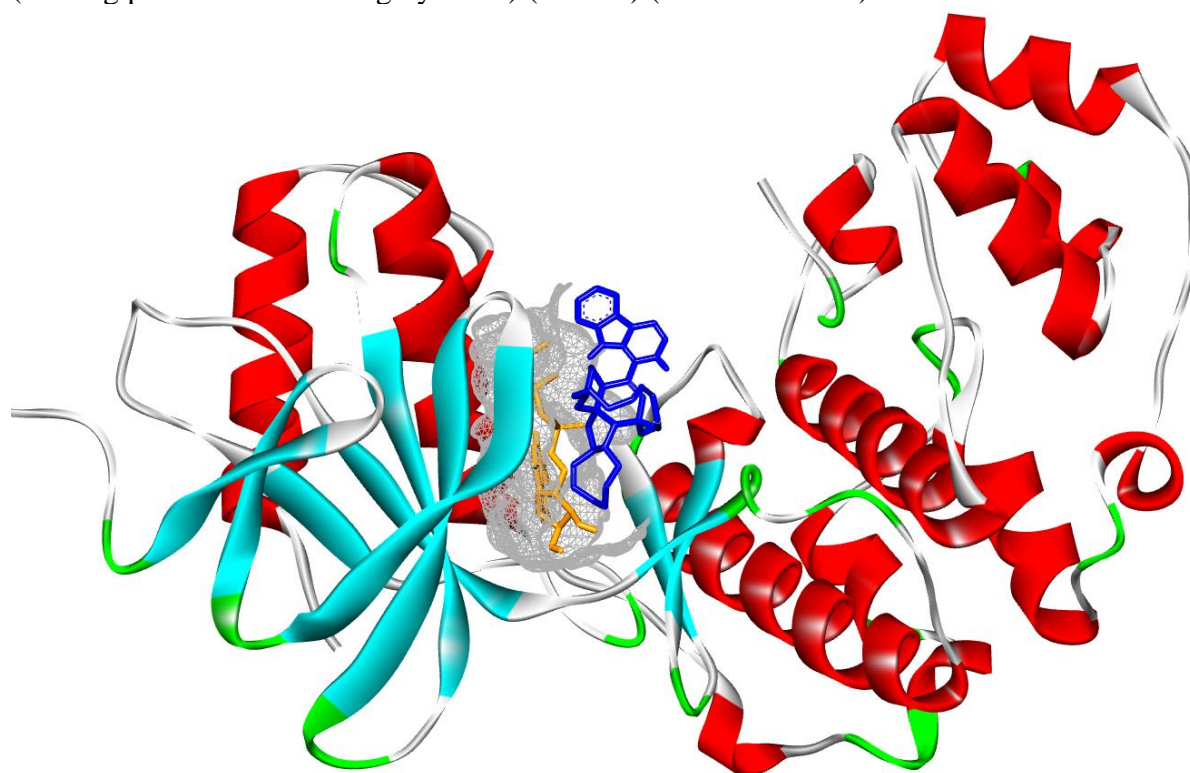
**Figure S19:** MZA (blue) and ATP (orange) is shown docked to mitogen-activated protein kinase (binding pocket is shown as gray mesh) (MAPK2/ERK2) (PDB ID: 2OJJ)



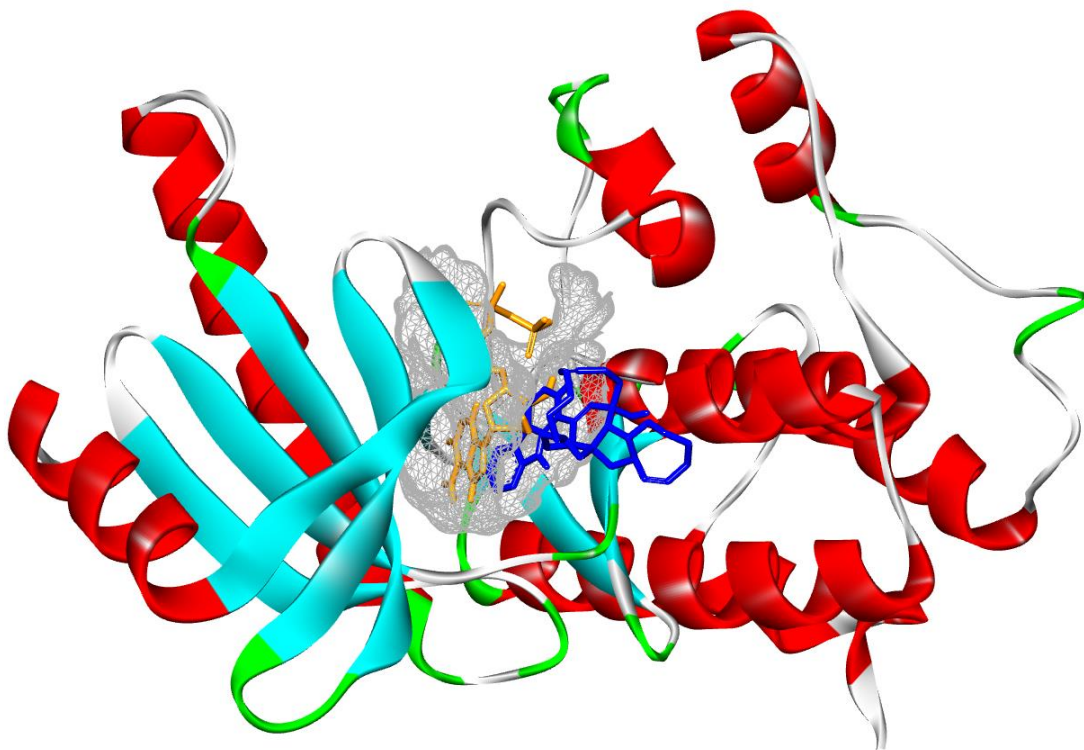
**Figure S20:** MZA (blue) and ATP (orange) is shown docked to NIMA-related protein kinase 2α (binding pocket is shown as gray mesh) (NEK2α) (PDB ID: 5M53)



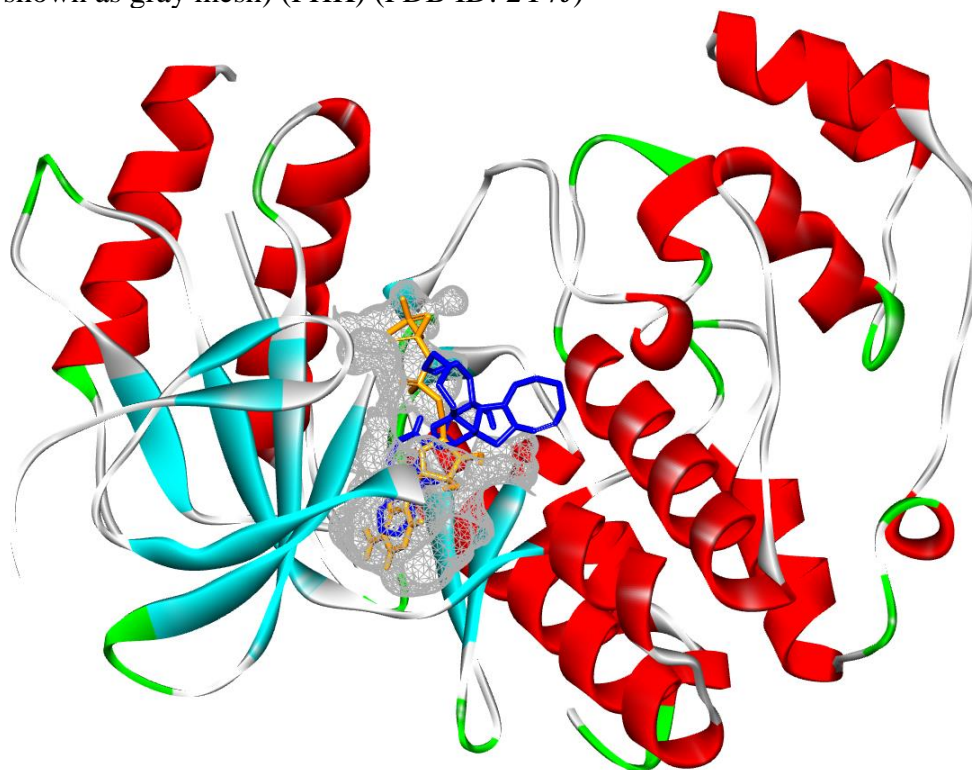
**Figure S21:** MZA (blue) and ATP (orange) is shown docked to AMP-activated protein kinase (binding pocket is shown as gray mesh) (AMPK) (PDB ID: 2YA3)



**Figure S22:** MZA (blue) and ATP (orange) is shown docked to stress-activated protein kinase-4 (binding pocket is shown as gray mesh) (SAPK4/P38δ) (PDB ID: 5ekn)

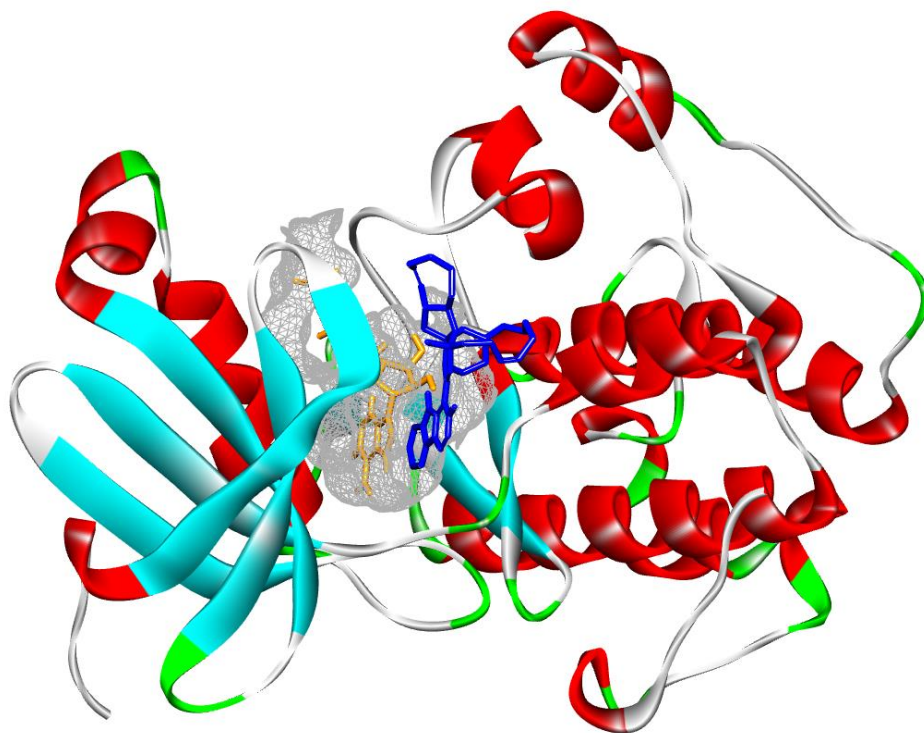


**Figure S23:** MZA (blue) and ATP (orange) is shown docked to phosphorylase kinase (binding pocket is shown as gray mesh) (PHK) (PDB ID: 2Y7J)

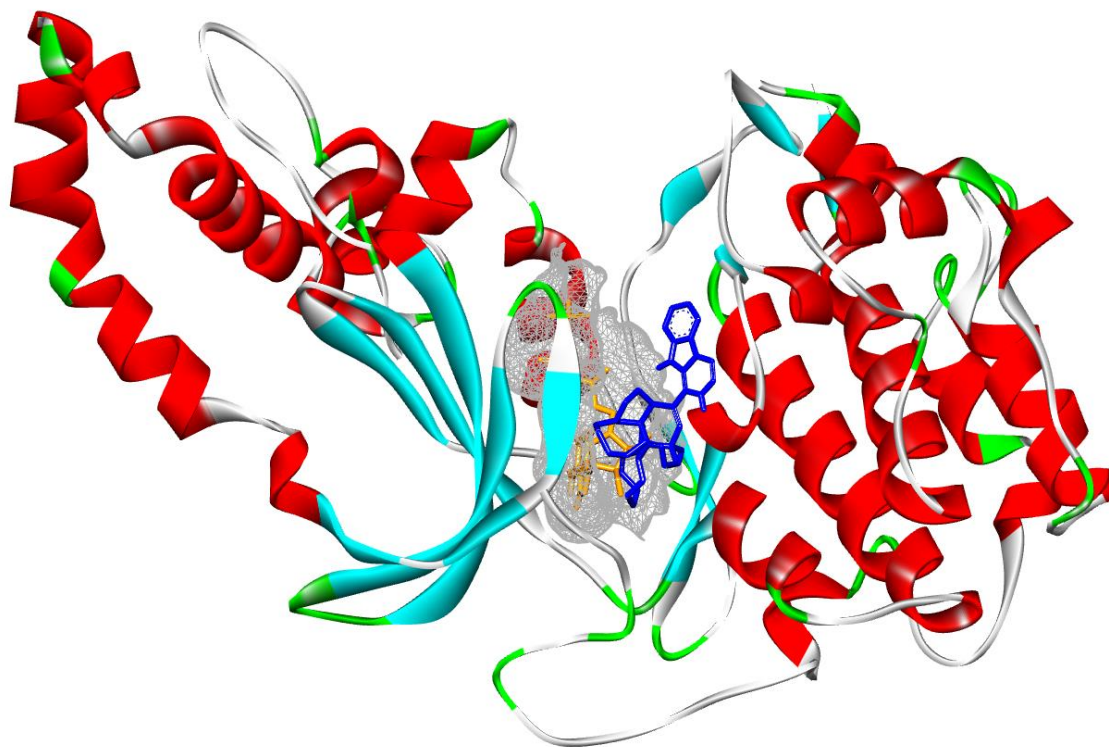


**Figure S24:** MZA (blue) and ATP (orange) is shown docked to stress-activated protein kinase-3 (binding pocket is shown as gray mesh) (SAPK3/P38 $\gamma$ ) (PDB ID: 1CM8)



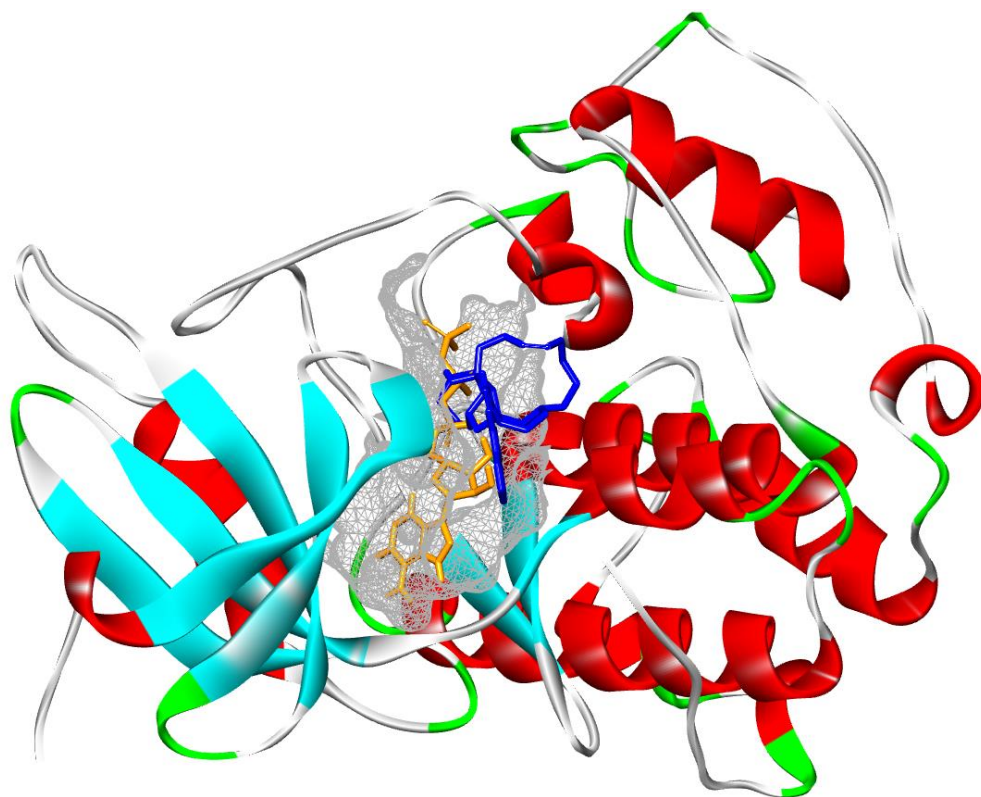


**Figure S25:** MZA (blue) and ATP (orange) is shown docked to 3-phosphoinositide-dependent protein kinase-1 (binding pocket is shown as gray mesh) (PDK1) (PDB ID: 5LVL)

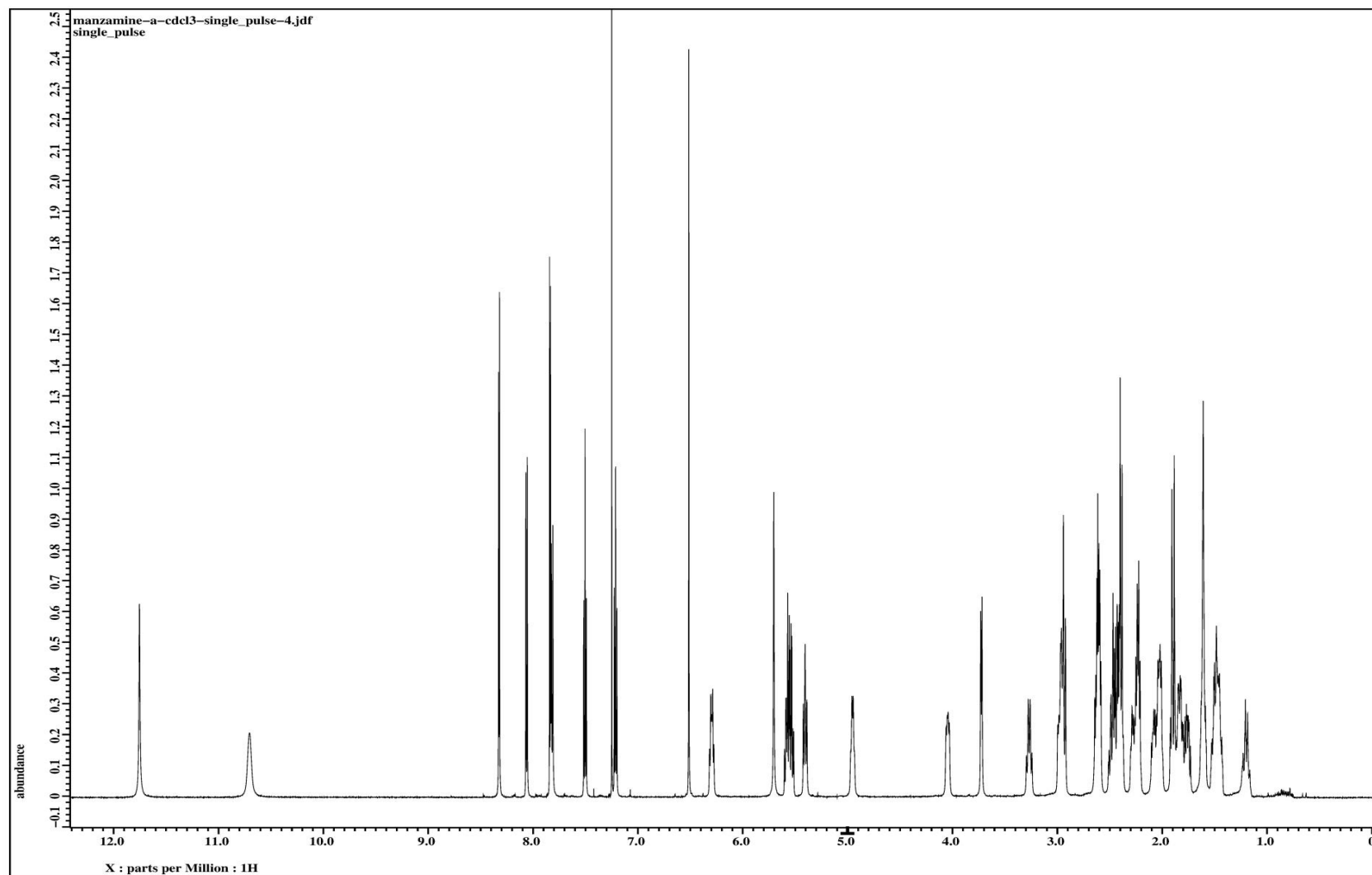


**Figure S26:** MZA (blue) and ATP (orange) is shown docked to Rho-dependent protein kinase (binding pocket is shown as gray mesh) (ROCK-II) (PDB ID: 6ED6)

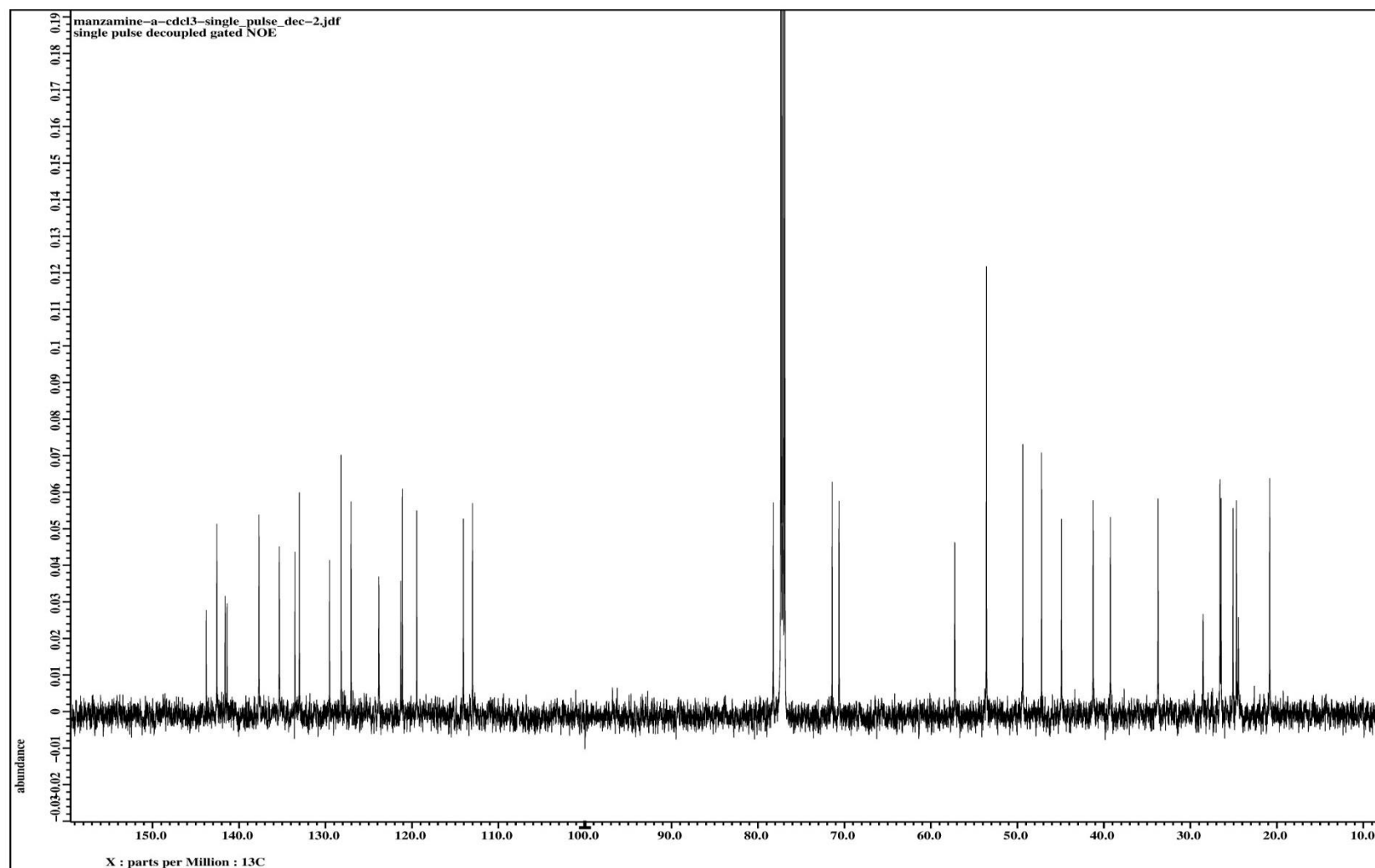




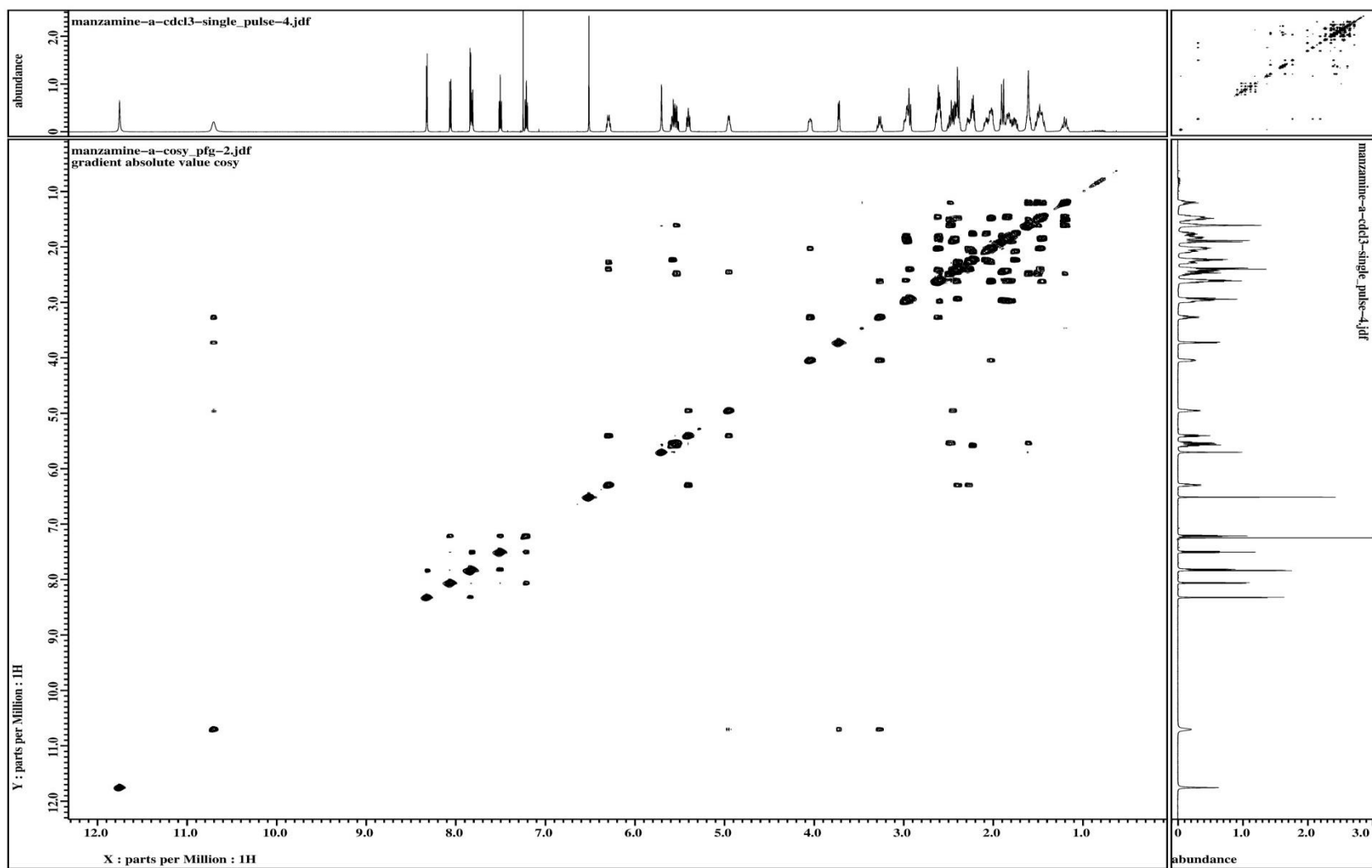
**Figure S27:** MZA (blue) and ATP (orange) is shown docked to cyclin-dependent kinase 2-cyclin A complex (binding pocket is shown as gray mesh) (CDK2/cyclin A) (PDB ID: 4EZ7)



**Figure S28.**  $^1\text{H}$  NMR of Manzamine A ( $\text{CDCl}_3$ , 600 MHz)

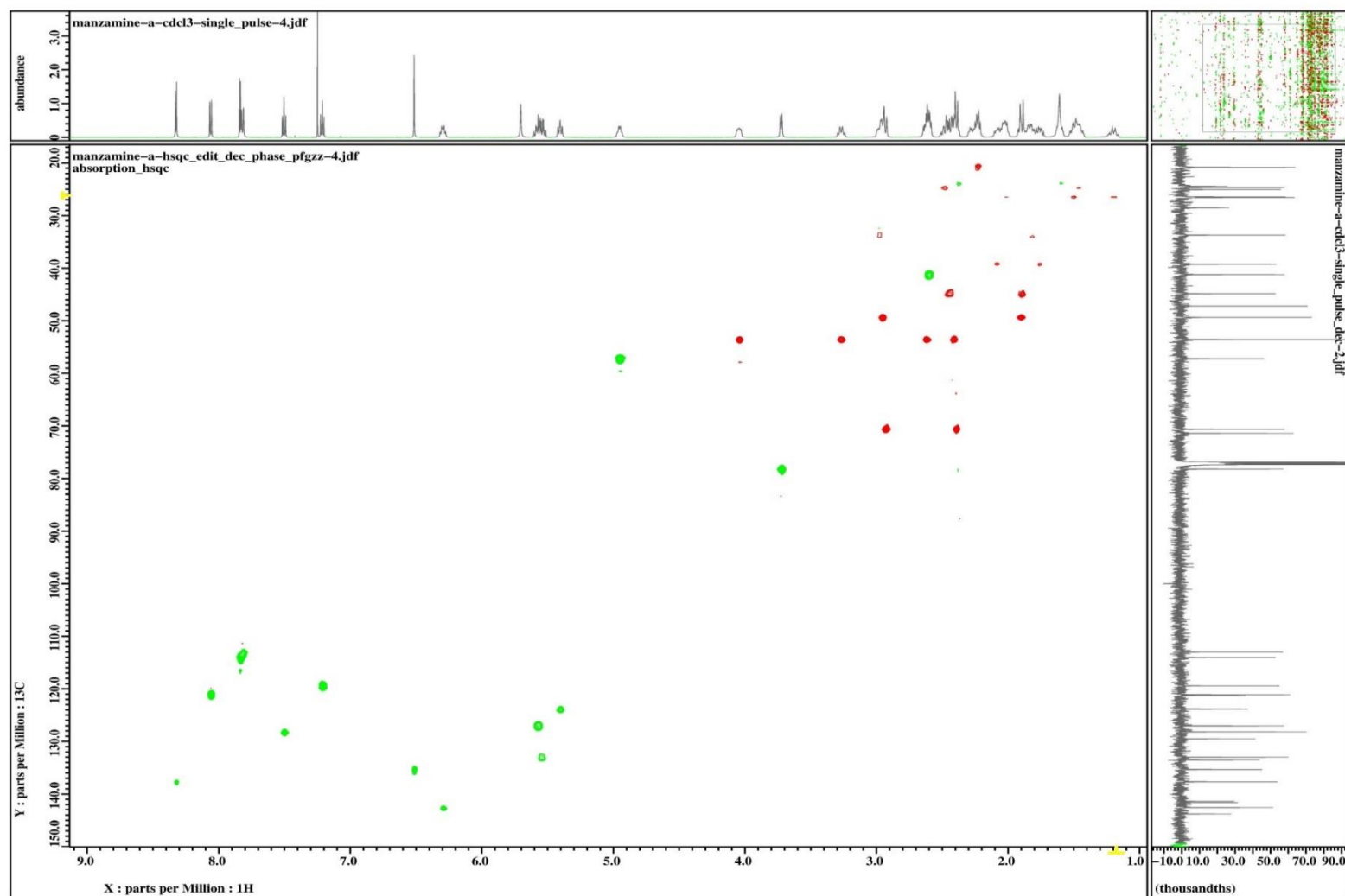


**Figure S29.**  $^{13}\text{C}$  NMR of Manzamine A ( $\text{CDCl}_3$ , 150 MHz)



**Figure S30.** 2D-COSY Spectrum Manzamine A ( $\text{CDCl}_3$ , 600 MHz)





**Figure S31.** 2D edited g-HSQC Spectrum of Manzamine A (CDCL<sub>3</sub>, 600 MHz)