Supplementary Information

**The nature of diamino linker and halogen bonding define selectivity of pyrrolopyrimidine based LIMK1 inhibitors.**

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**1-(3-chlorophenyl)-2-cyano-3-(2-((5-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)amino)ethyl)guanidine (8).**

1H NMR (DMSO-d6, 400 MHz)Shape

Description automatically generated with medium confidence

13C NMR (DMSO-d6, 100 MHz)

Shape

Description automatically generated with medium confidence

Analytical HPLC trace



HRMS ESI+ spectra



**1-(3-bromophenyl)-2-cyano-3-(2-((5-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)amino)ethyl)guanidine (9).**

1H NMR (MeOD, 400 MHz)

Shape

Description automatically generated with medium confidence

13C NMR (MeOD, 125 MHz)

Shape

Description automatically generated with medium confidence

Analytical HPLC trace



HRMS ESI+ spectra



**2-cyano-1-(3-iodophenyl)-3-(2-((5-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)amino)ethyl)guanidine (10).**

1H NMR (MeOD, 400 MHz)

Shape

Description automatically generated with low confidence

13C NMR (MeOD, 100 MHz)

Shape

Description automatically generated with medium confidence

Analytical HPLC trace



HRMS ESI+ spectra



**1-(3-chlorophenyl)-2-cyano-3-(3-((5-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)amino)propyl)guanidine (11).**

1H NMR (MeOD, 400 MHz)

Shape

Description automatically generated with low confidence

13C NMR (MeOD, 100 MHz)

Shape

Description automatically generated with medium confidence

Analytical HPLC trace



HRMS ESI+ spectra



**1-(3-bromophenyl)-2-cyano-3-(3-((5-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)amino)propyl)guanidine (12).**

1H NMR (MeOD, 400 MHz)

Shape

Description automatically generated with medium confidence

13C NMR (MeOD, 100 MHz)

Shape

Description automatically generated with medium confidence

Analytical HPLC trace



HRMS ESI+ spectra



**2-cyano-1-(3-iodophenyl)-3-(3-((5-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)amino)propyl)guanidine (13).**

1H NMR (MeOD, 400 MHz)

Shape

Description automatically generated with medium confidence

13C NMR (MeOD, 100 MHz)

Shape

Description automatically generated with medium confidence

Analytical HPLC trace



HRMS ESI+ spectra



**1-(3-chlorophenyl)-2-cyano-3-(4-((5-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)amino)butyl)guanidine (14).**

1H NMR (MeOD, 400 MHz)

Shape

Description automatically generated with medium confidence

13C NMR (MeOD, 100 MHz)

Shape

Description automatically generated with medium confidence

Analytical HPLC trace



HRMS ESI+ spectra



**1-(3-bromophenyl)-2-cyano-3-(4-((5-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)amino)butyl)guanidine (15).**

1H NMR (MeOD, 400 MHz)

Shape

Description automatically generated with medium confidence

13C NMR (MeOD, 100 MHz)

Shape

Description automatically generated with medium confidence

Analytical HPLC trace



HRMS ESI+ spectra



**2-cyano-1-(3-iodophenyl)-3-(4-((5-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)amino)butyl)guanidine (16).**

1H NMR (MeOD, 400 MHz)

Shape

Description automatically generated with medium confidence

13C NMR (MeOD, 125 MHz)

Shape

Description automatically generated with medium confidence

Analytical HPLC trace



HRMS ESI+ spectra



**1-(3-chlorophenyl)-2-cyano-3-((1*r*,4*r*)-4-((5-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)amino)cyclohexyl)guanidine (17).**

1H NMR (MeOD, 400 MHz)

Shape

Description automatically generated with medium confidence

13C NMR (MeOD, 125 MHz)

Shape

Description automatically generated with medium confidence

Analytical HPLC trace



HRMS ESI+ spectra



**1-(3-bromophenyl)-2-cyano-3-((1*r*,4*r*)-4-((5-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)amino)cyclohexyl)guanidine (18).**

1H NMR (MeOD, 400 MHz)

Shape

Description automatically generated with medium confidence

13C NMR (MeOD, 100 MHz)

Shape

Description automatically generated with medium confidence

Analytical HPLC trace



HRMS ESI+ spectra



**2-cyano-1-(3-iodophenyl)-3-((1*r*,4*r*)-4-((5-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)amino)cyclohexyl)guanidine (19).**

1H NMR (MeOD, 400 MHz)

Shape

Description automatically generated with medium confidence

13C NMR (MeOD, 125 MHz)

Shape

Description automatically generated with medium confidence

Analytical HPLC trace



HRMS ESI+ spectra



**(*S*)-*N*'-cyano-*N*-(3-iodophenyl)-2-methyl-4-(5-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)piperazine-1-carboximidamide (22).**

1H NMR (MeOD, 400 MHz)

Shape

Description automatically generated with medium confidence

13C NMR (MeOD, 125 MHz)

Shape

Description automatically generated with medium confidence

Analytical HPLC trace



HRMS ESI+ spectra



**1-(3-bromophenyl)-2-cyano-3-((1*R*,2*R*)-2-((5-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)amino)cyclohexyl)guanidine (23).**

1H NMR (MeOD, 400 MHz)

Shape

Description automatically generated with medium confidence

13C NMR (MeOD, 100 MHz)

Shape

Description automatically generated with medium confidence

Analytical HPLC trace



HRMS ESI+ spectra



**2-cyano-1-(3-iodophenyl)-3-((1*R*,2*R*)-2-((5-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)amino)cyclohexyl)guanidine (24).**

1H NMR (MeOD, 400 MHz)

Shape

Description automatically generated with medium confidence

13C NMR (MeOD, 125 MHz)

Shape

Description automatically generated with medium confidence

Analytical HPLC trace



HRMS ESI+ spectra



**1-(3-bromophenyl)-2-cyano-3-((1*S*,2*S*)-2-((5-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)amino)cyclohexyl)guanidine (25).**

1H NMR (MeOD, 400 MHz)

Shape

Description automatically generated with medium confidence

13C NMR (MeOD, 100 MHz)

Shape

Description automatically generated with medium confidence

Analytical HPLC trace



HRMS ESI+ spectra



**2-cyano-1-(3-iodophenyl)-3-((1*S*,2*S*)-2-((5-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)amino)cyclohexyl)guanidine (26).**

1H NMR (MeOD, 400 MHz)

Shape

Description automatically generated with medium confidence

13C NMR (MeOD, 100 MHz)

Shape

Description automatically generated with medium confidence

Analytical HPLC trace



HRMS ESI+ spectra



Diagram

Description automatically generated

**Fig S1.** Comparison of LIMK1 crystal structure of 5NXC ligand (green) vs predicted binding pose (yellow). RMSD = 1.17 Å

Diagram

Description automatically generated

**Fig S2.** Comparison of LIMK1 crystal structure of 4TPT ligand (green) vs predicted binding pose (yellow). RMSD = 1.17 Å

**Table S1.** GLIDE score of compounds **1, 24** and **26** docking with LIMK1 and LIMK2

|  |  |  |
| --- | --- | --- |
| Compounds | LIMK1 | LIMK2 |
|  | –8.52 | –5.02 |
|  | –4.92 | –3.78 |
|  | –5.43 | –2.44 |