Electronic Supplementary Information

Synthesis of benzimidazole/triphenylamine-based compounds, their evaluation of bioactivities and insilico study with receptor tyrosine kinases

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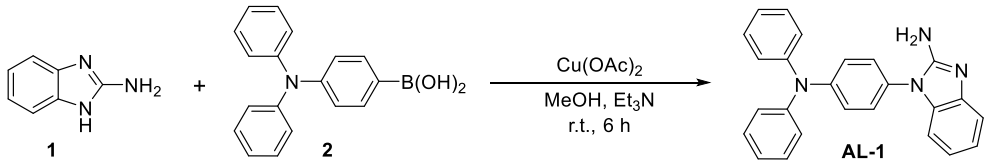
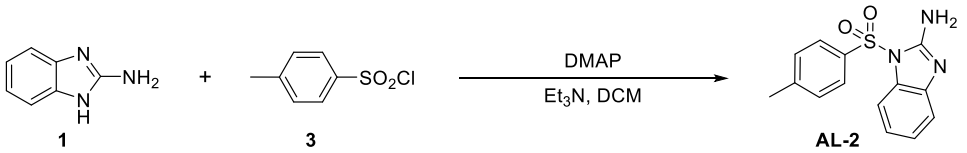
Experimental procedure of AL-1



The combination of copper acetate and the base system was used to produce **AL-1** by reacting 2-aminobenzimidazole **1** with 4-(diphenylamino)phenylboronic acid **2**.

2-Aminobenzimidazole **1** (1.0 mmol) was dissolved in methanol (4 mL) and mixed well. Et_3N (2.0 mmol) was added and followed by copper acetate (0.5 mmol, 0.5 equiv.), and then 4-(diphenylamino)phenylboronic acid **2** (1.1 mmol) was added. The contents were stirred at room temperature (r.t., 25 ℃) for 6 hours until the reaction substrate consumed utterly. The progress of the reaction was monitored by thin-layer chromatography (TLC). After the reaction was finished, the reaction mixture was extracted with ethyl acetate, neutralized with $NaHCO_3$, and washed with brine solution. After the organic layer was dried over anhydrous Na_2SO_4. Column chromatography was used to purify the desired product **AL-1**.

Experimental procedure of AL-2



The **AL-2** was synthesized using a simple sulfonylation reaction as the reported method in the literature.[1]

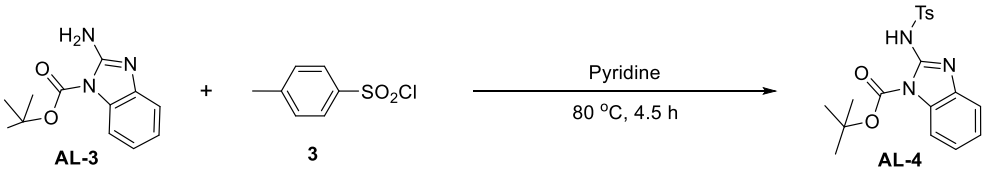
The mixture of 1.25 mmol (1.25 equiv.) of *p*-toluenesulfonyl chloride (**3**), 0.4 mmol (0.4 equiv.) of 4-dimethylaminopyridine (4-DMAP) and 1.25 mmol (1.25 equiv.) of Et_3N as catalysts were added in 15 mL of dry CH_2Cl_2 at room temperature (25 ℃) and under N_2 atmosphere. After complete dissolving of the mixture, 1.0 mmol (1.0 equiv.) of 2-amino-benzimidazole **1** was added and mixed well. The progress of the reaction was monitored by TLC. After the reaction was finished, the reaction mixture was neutralized with Na_2CO_3, and

washed with brine. After the organic layer was dried over anhydrous Na_2SO_4, the solvent was evaporated. Finally, recrystallization was carried out using methanol as a solvent to get **AL-2**.

Experimental procedure of AL-3

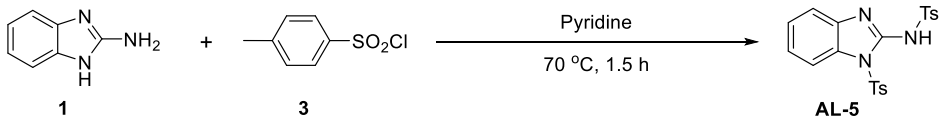
According to the reported procedure,[2] compound **AL-3** was synthesized. The solution containing 1.0 mmol of **1** (1.0 equiv.), 1.0 mmol of di-tert-butyl dicarbonate (1.0 equiv.), and triethylamine (0.6 mL, 4.37 mmol) in dry THF (2.4 mL) was allowed to stir well at room temperature (25 ℃) for 5 hours. Then, the organic layer of reaction crude portioned between AcOEt (20 mL) and $NaHCO_3$ saturated solution (20 mL) was extracted with brine (20 mL). The obtained organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. From typical silica gel flash chromatography, **AL-3** was obtained with good purity.

Experimental procedure of AL-4



Boc protected aminobenzimidazole, **AL-3** (1.0 mmol, 1.0 equiv.**)** was dissolved in 2 mL of pyridine. After adding 2.0 mmol of **3** (2.0 equiv.), the mixture was stirred well at 80 ℃ for 4.5 hours. After the consumption of the initial reactants, the reaction was stopped by adding water to give white solid. After a filtration, the resulting solid was recrystallised with aqueous ethanol to give the desired compound **AL-4**.

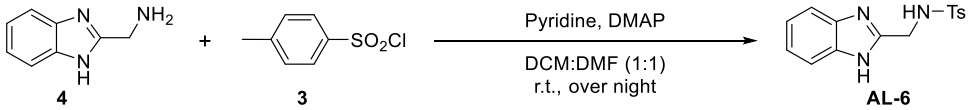
Experimental procedure of AL-5



1 3 AL-5

A solution of **1** (3.0 mmol, 1.0 equiv.) and **3** (9.0 mmol, 3.0 equiv.) in anhydrous pyridine (10 mL) was stirred at 70 ℃ for 1.5 hours. The reaction was stopped and the reaction mixture was allowed to cool to room temperature (25 ℃). The distilled ice water was poured into reaction mixture until the precipitate was formed. After a filtration, the resulting solid was recrystallised with aqueous ethanol to give the desired compound **AL-5**.

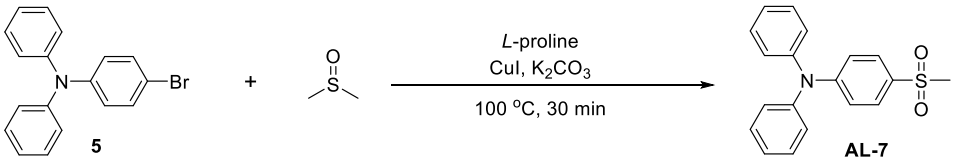
Experimental procedure of AL-6



4 3 AL-6

(1*H*-benzo[d]imidazol-2-yl)methanamine **4** (2.0 mmol, 1.0 equiv.), pyridine (10 mmol, 5.0 equiv.), and 4-DMAP (0.2 mmol, 0.1 equiv.) were dissolved in 2 mL mixed solvent of DCM:DMF (1:1) and mixed well at 0 °C. The solution of **3** (2.2 mmol, 1.1 equiv.) in 2 mL of DCM:DMF (1:1) was added drop by drop to the above solution. The resulting mixture was stirred at room temperature (25 ℃) overnight. After the completion of the reaction, the reaction mixture was quenched with H_2O (15 mL) and extracted with ethylacetate (3 × 15 mL). Then, the organic layer washed with brine solution and dried over anhydrous Na_2SO_4. After filtration and evaporation of the solvents under reduced pressure, the desired product was purified by preparatory column chromatography wth PE : EA (1:2) to give the desired compound **AL-6**.

Experimental procedure of AL-7



According to the reported procedure,[3] **AL-7** was formed by the reaction between 4-bromo-*N*,*N*-diphenylaniline (**5**) and DMSO using a catalytic system containing CuI, K_2CO_3, and *L*-proline.

Under a N_2 atmosphere, 4-bromo-*N*,*N*-diphenylaniline **5** (2.0 mmol, 1.0 equiv.), DMSO (2.8 mmol, 1.4 equiv.), CuI (0.05 mmol.), K_2CO_3 (1.0 equiv.), and *L*-proline were added to a Schlenk tube. The resulting mixture was allowed to stir for 30 minutes at 100 ℃. After the completion of the reaction, the reaction mixture was quenched with H_2O (15 mL) and extracted with ethylacetate (3 × 15 mL). The organic layers were combined and washed with brine solution, dried over anhydrous Na_2SO_4. After filtration and evaporation of the solvents under reduced pressure, the crude product was purified by column chromatography on silica gel to afford the desired product **AL-7**.

NMR Spectra for All Compounds AL-1 ~ AL-7

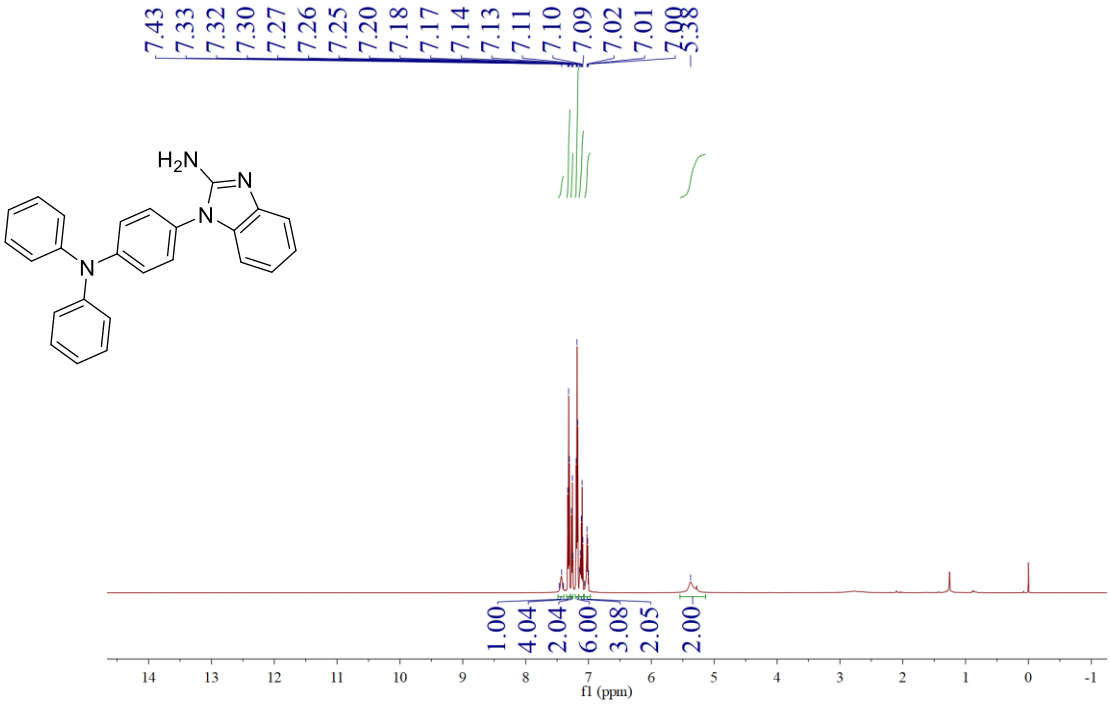


Figure S1. ^{1}H NMR spectrum of **AL-1**.

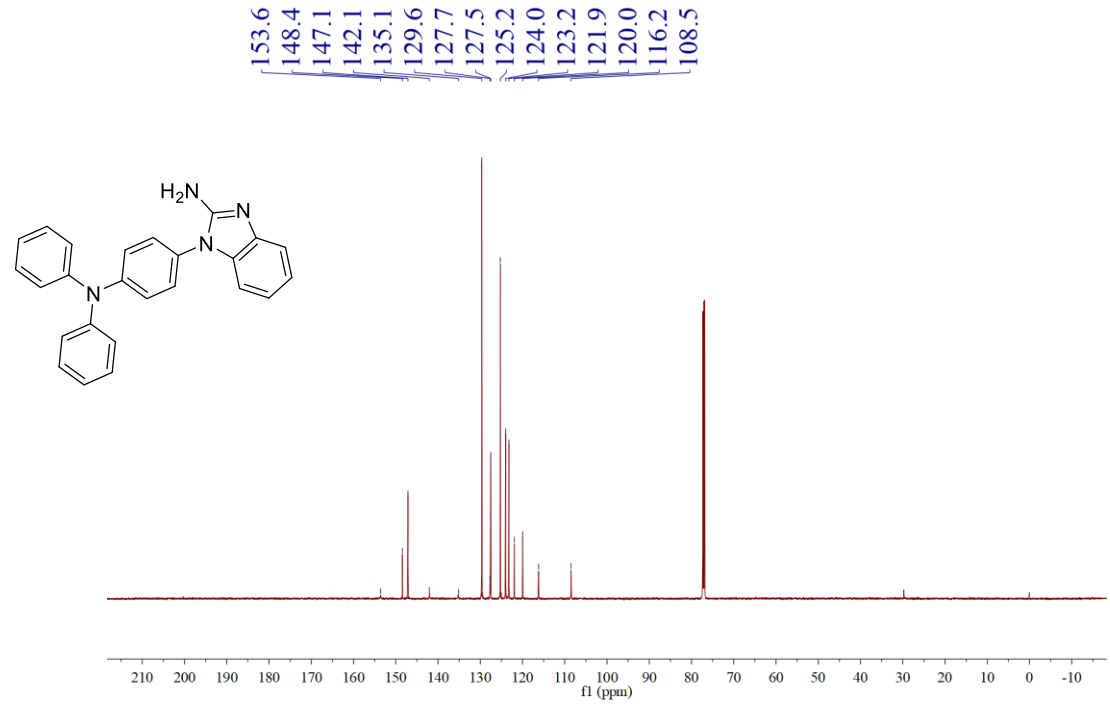


Figure S2. ^{13}C NMR spectrum of **AL-1**.

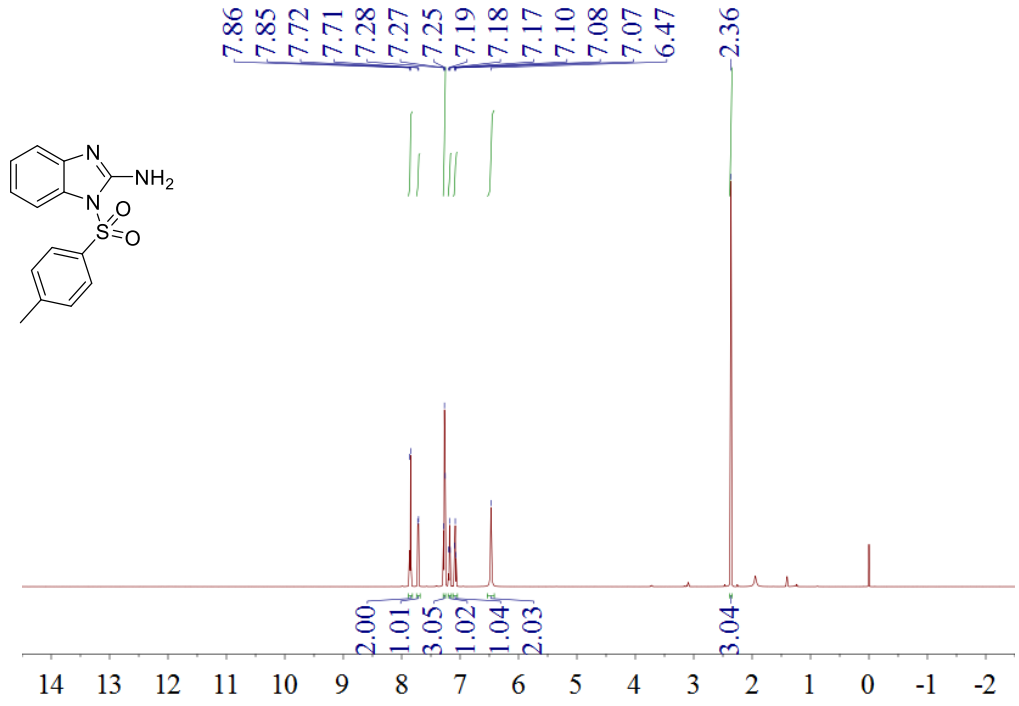


Figure S3. ^{1}H NMR spectrum of **AL-2**.

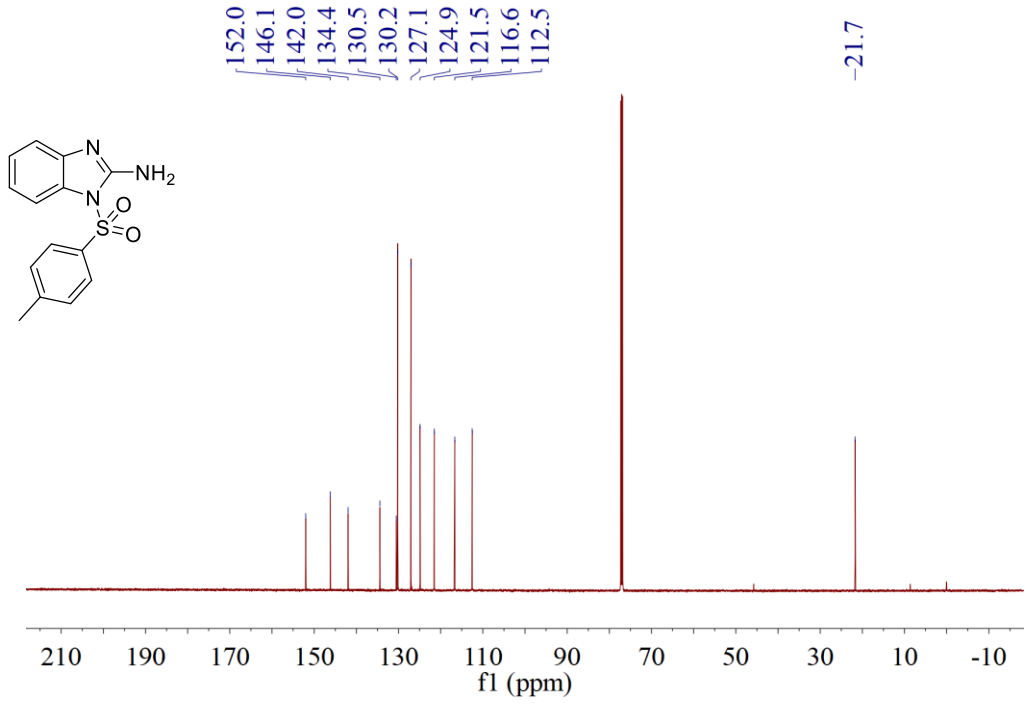


Figure S4. ^{13}C NMR spectrum of **AL-2**.

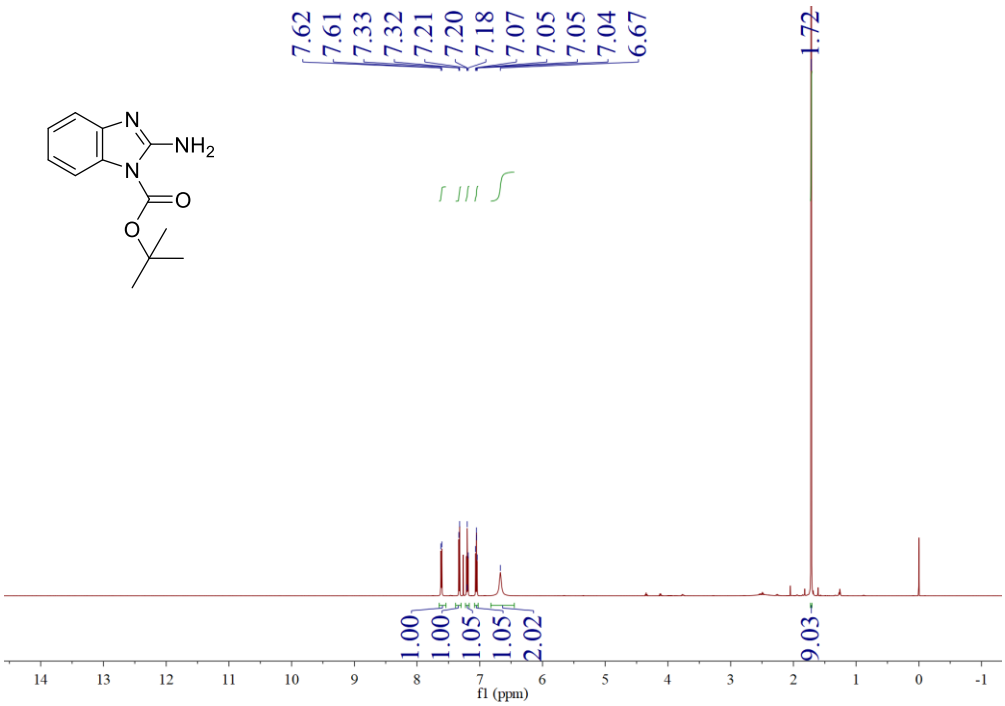


Figure S5. ^{1}H NMR spectrum of **AL-3**.

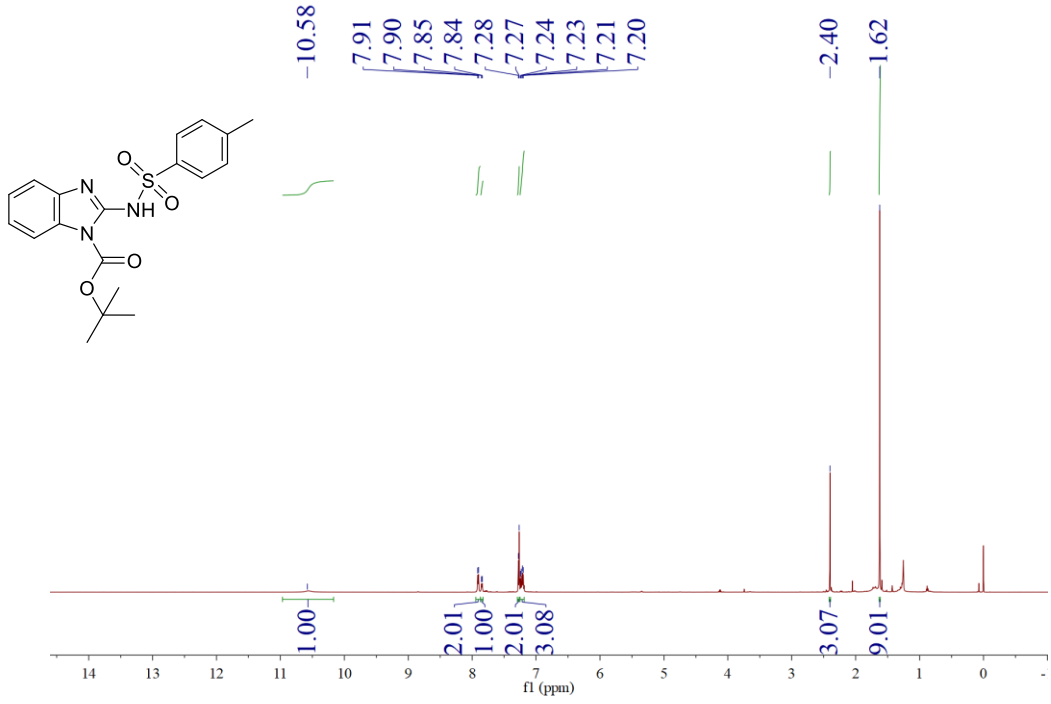


Figure S6. ^{1}H NMR spectrum of **AL-4**.

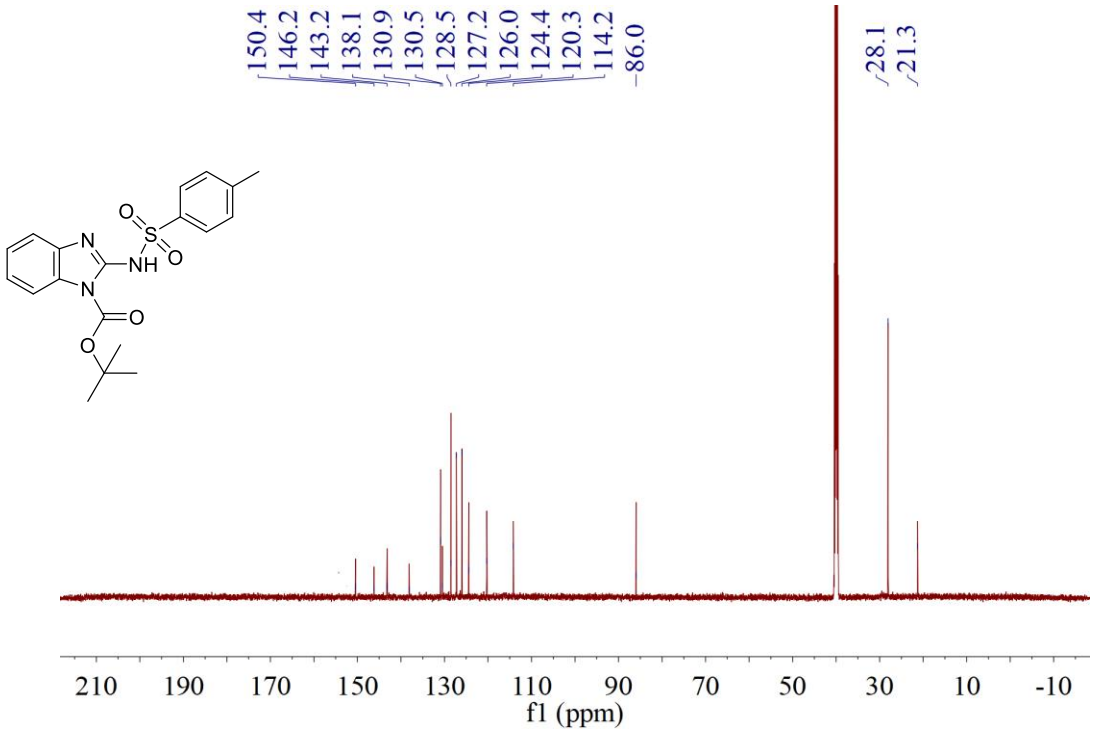


Figure S7. ^{13}C NMR spectrum of **AL-4**.

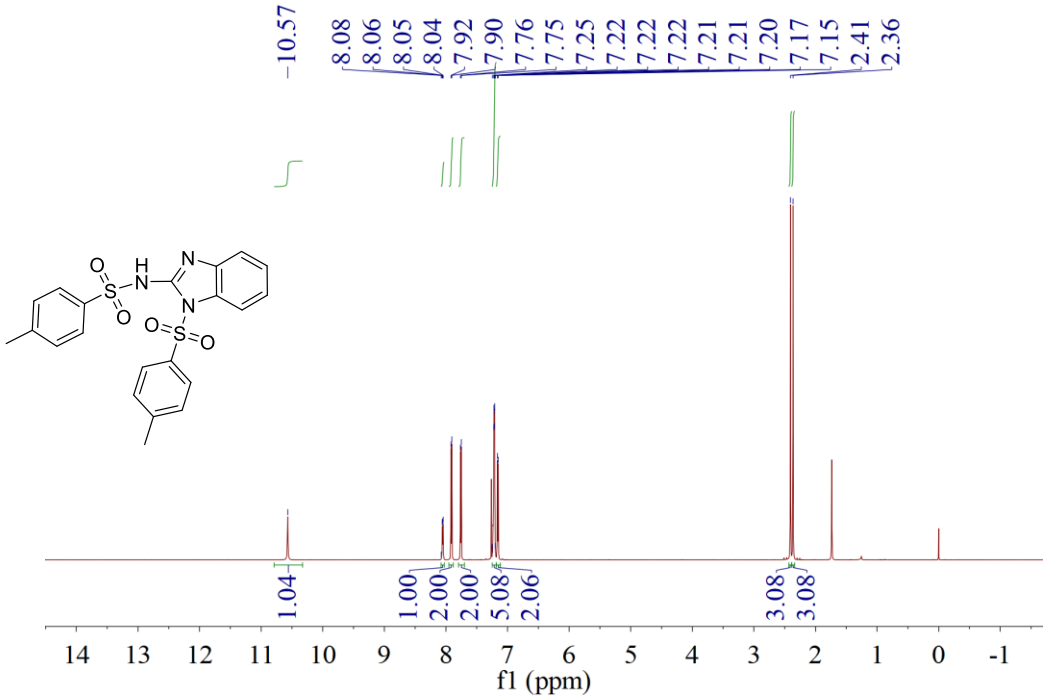


Figure S8. ^{1}H NMR spectrum of **AL-5**.

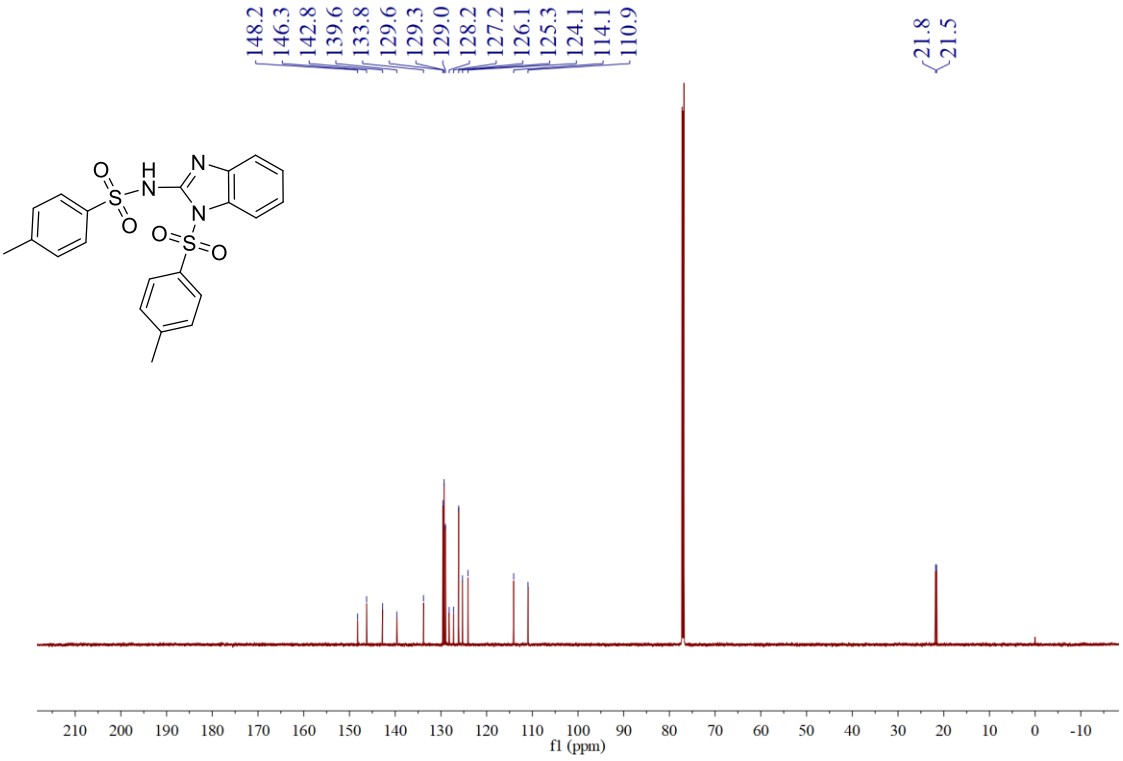


Figure S9. ^{13}C NMR spectrum of **AL-5**.

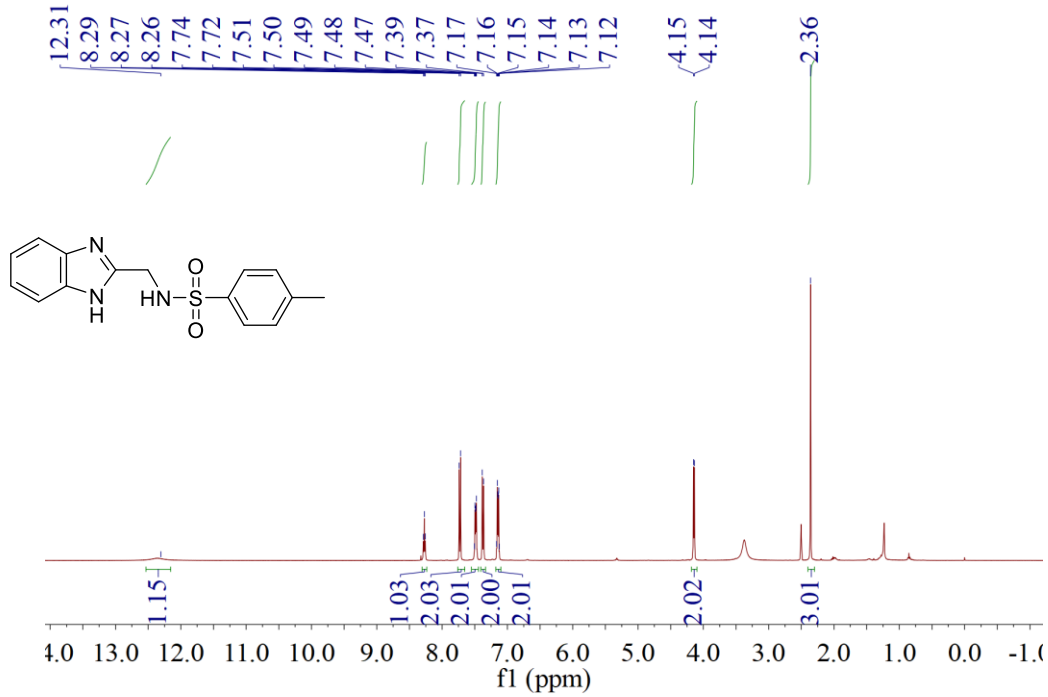


Figure S10. ^{1}H NMR spectrum of **AL-6**.

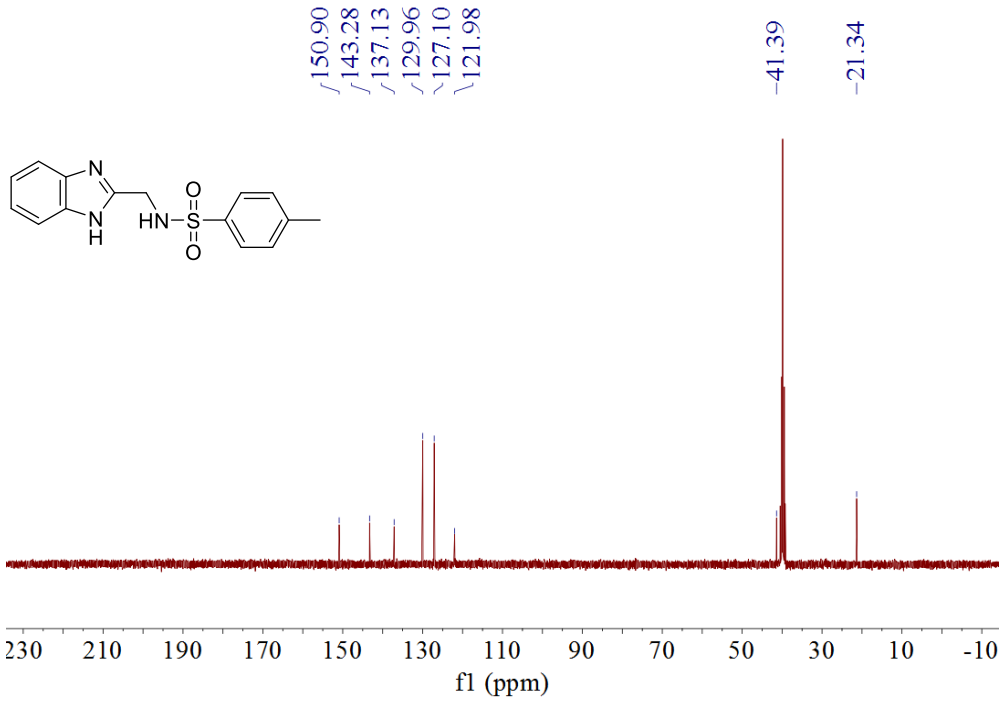


Figure S11. ^{13}C NMR spectrum of **AL-6**.

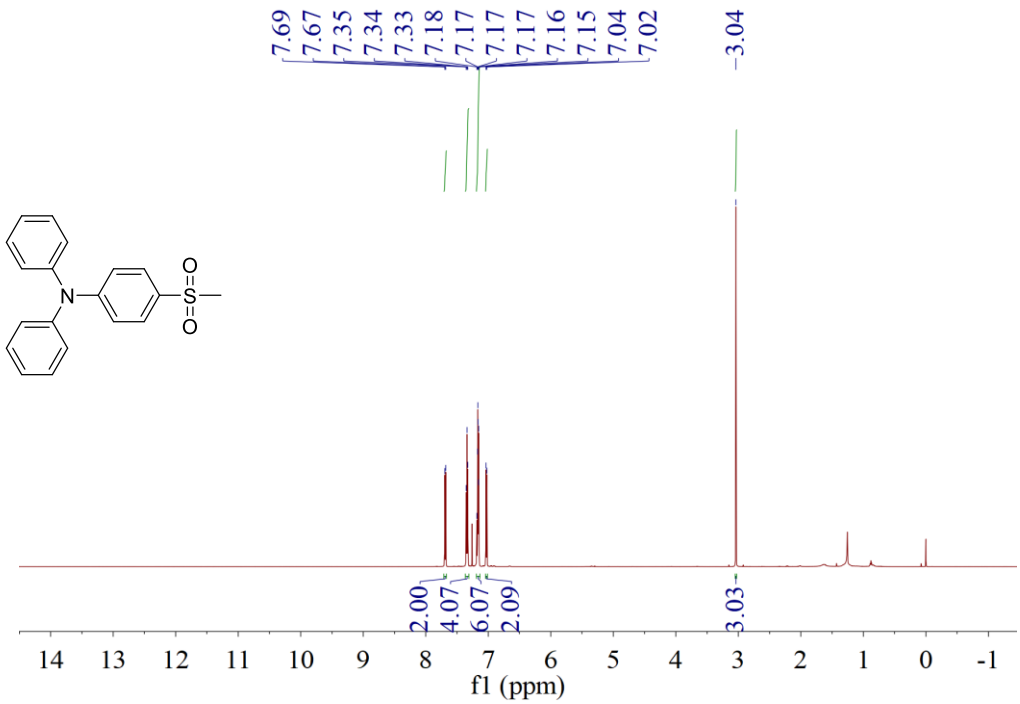


Figure S12. ^{1}H NMR spectrum of **AL-7**.

Table S1. The value of $logIC_{50}$ and regression coefficient (R^2) associated with the first reduction potential of the synthesized compounds.

Comp.	First reduction potential (V)	$logIC_{50}$				
		A549	**H446**	**SPC-A-1**	**MCF-7**	**HepG2**
AL-1	1.10	1.4158	1.3953	1.1607	1.1934	1.2455
AL-2	0.96	2.0000	1.8584	1.9051	2.0000	2.0000
AL-3	0.93	2.0000	2.0000	2.0000	2.0000	2.0000
AL-4	1.05	2.0000	2.0000	1.9956	2.0000	2.0000
AL-5	1.01	1.8600	1.7594	2.0000	2.0000	2.0000
AL-6	0.8	2.0000	2.0000	2.0000	2.0000	2.0000
AL-7	0.82	2.0000	1.8674	1.8688	2.0000	1.9544
R^2	-	0.4065	0.3462	0.2666	0.3326	0.3023

Table S2. The ADME properties (drug-likeness) properties of **AL-1** and the selected PBI-based ligands.

Comp.	MW	DonorHB	AccptHB	QPlogPo/w	QPP MDCK	CNS	Rule of Five
OMe-PBI	224.26	0	2.25	3.43	2935.19	1	0
1PBI	194.23	0	1.5	3.36	2926.66	1	0
OH-PBI	210.23	1	2.25	2.59	810.51	0	0
AL-1	376.46	2	2.5	5.88	957.85	0	1

The structures of the selected PBI-based ligands can be seen in **Figure S13** in the following; **MW** = molecular weight; **DonorHB** = number of donor hydrogen bonds; **AccptHB** = number of acceptor hydrogen bonds; **QPlogPo/w** = Predicted octanol/water partition coefficient; **QPPMDCK** = predicted apparent MDCK cell permeability in nm/sec; **CNS** = Predicted central nervous system activity on a -2 (inactive) to +2 (active); **Rule of Five** = Number of violations of Lipinski's rule of five.

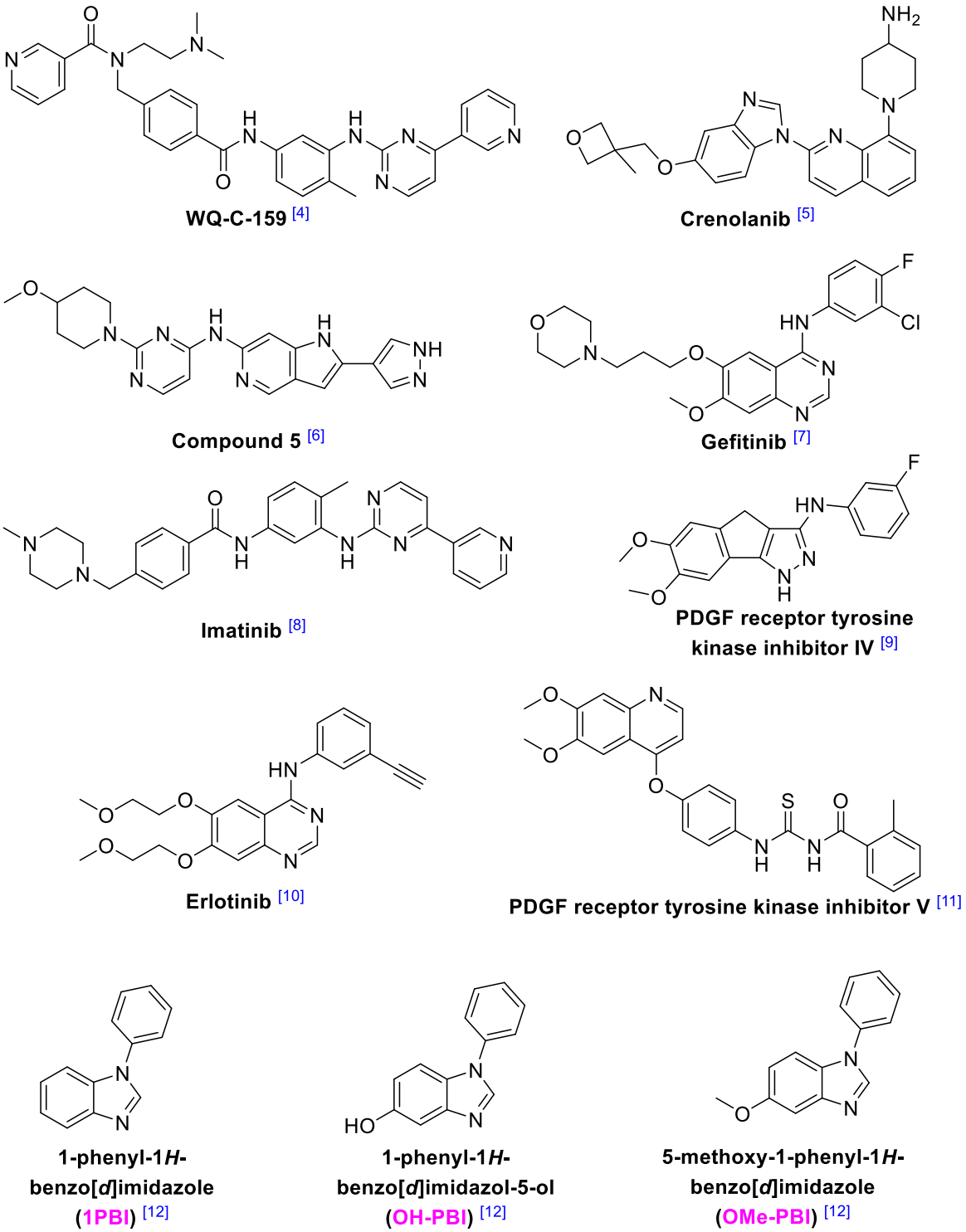


Figure S13. Structures of the docked compounds.

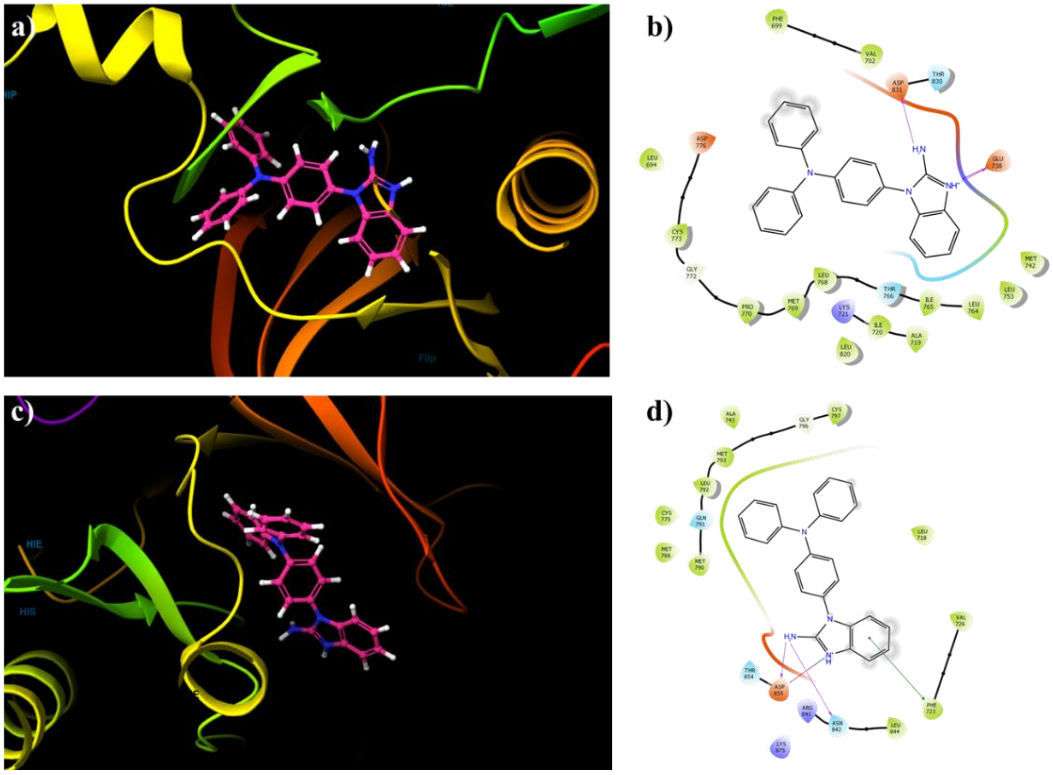


Figure S14. 3D and 2D of docked pose for the interaction of **AL-1** with the WT EGFR (1M17) (a, b) and the mutant EGFR (4RJ5) (c, d).

Table S3. The binding free energies, ΔG bind (kcal/mol) of the **AL-1** and the selected compounds against RTKs calculated in MM-GBSA. [a]

RTKs (PDB Code)	PDGFRα WT (5GRN)	PDGFRα_T674I (6JOI)	EGFR WT (1M17)	EGFR_T790M / L858R (4RJ5)
		Tested ligands		
Crenolanib	-98.50	-57.37	-44.90	-55.45
Erlotinib	-	-	-58.63	-48.98
Gefitinib	-	-	-53.77	-40.56
Imatinib	-59.60	-54.48	-54.87	-51.40
PDGF receptor tyrosine kinase inhibitor IV [b]	-52.02	-53.50	-	-
PDGF receptor tyrosine kinase inhibitor V [b]	-51.11	-35.06	-	-
OMe-PBI	-28.37	-44.69	-45.91	-38.57
1PBI	-98.50	-42.24	-41.60	-36.00
OH-PBI	-66.63	-43.51	-41.74	-34.90
AL-1	**-79.89**	**-43.58**	**-51.85**	**-39.99**
AL-1 (post dynamics)	-62.73 ±1.86	-61.44 ±1.30	-58.82 ±0.93	-51.02 ±1.41

[a] The structures of the selected compounds can be seen in **Figure S13**.

[b] Inhibit PDGFR α and β.

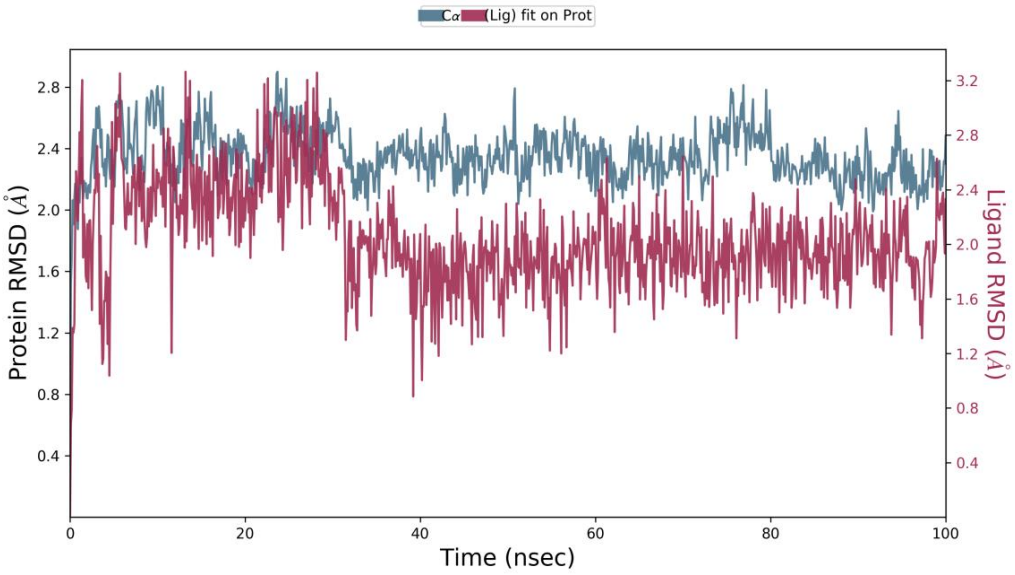


Figure S15. MD simulation pose for the complex containing WT PDGFRα (5GRN) and **AL-1**.

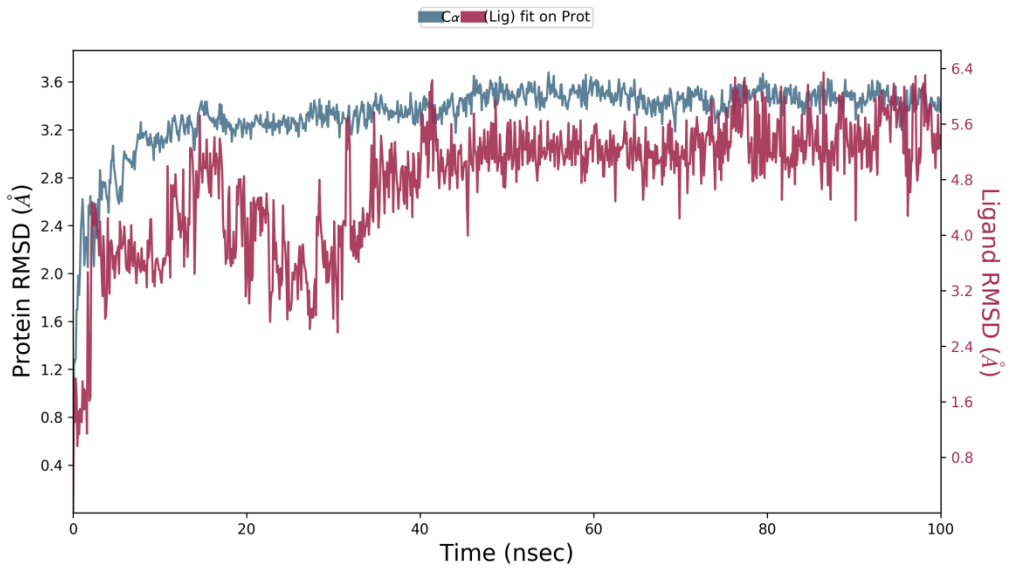


Figure S16. MD simulation pose for the complex containing mutant PDGFRα (6JOI) and **AL-1**.

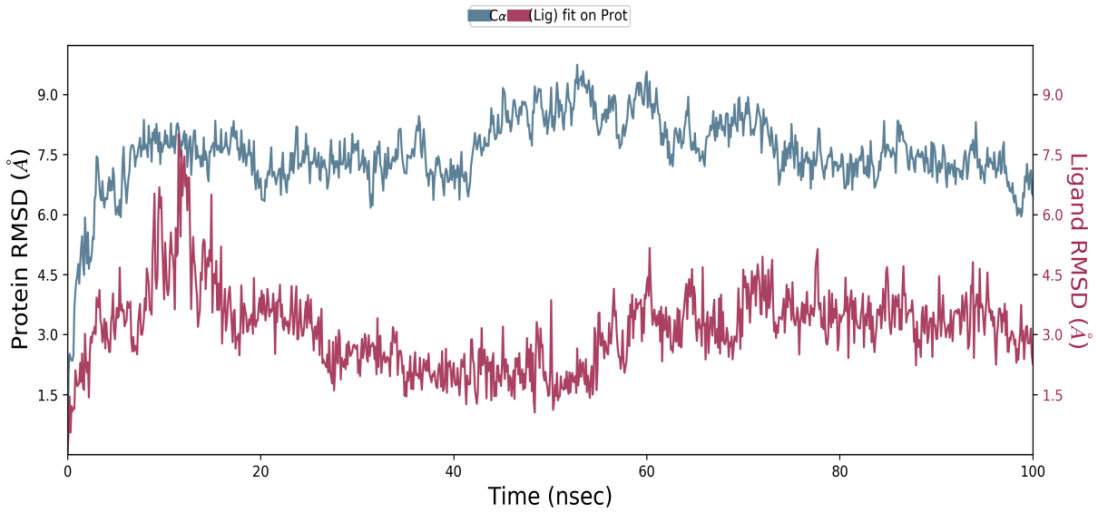


Figure S17. MD simulation pose for the complex containing WT EGFR (1M17) and **AL-1**.

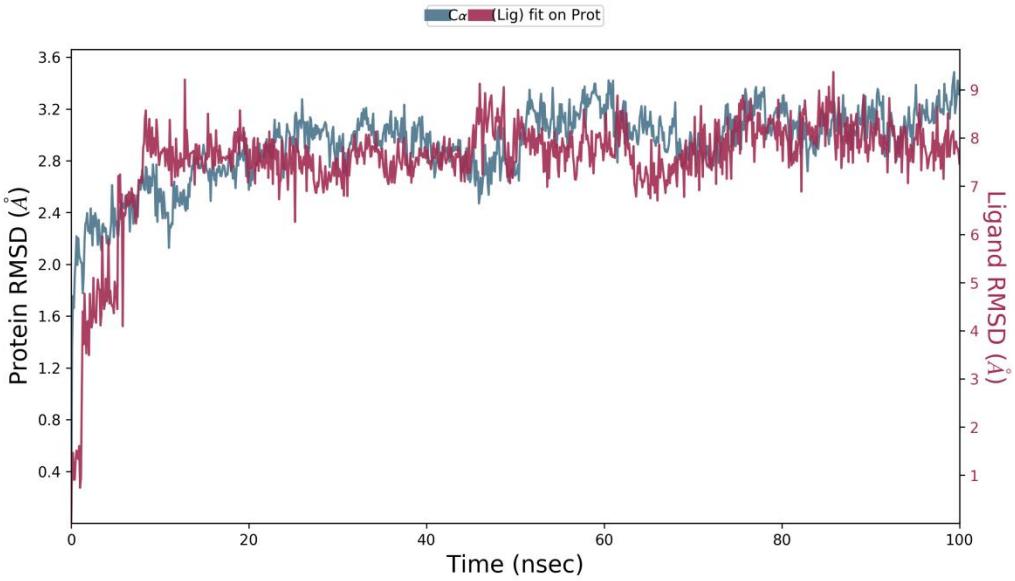


Figure S18. MD simulation pose for the complex containing mutant EGFR (4RJ5) and **AL-1**.

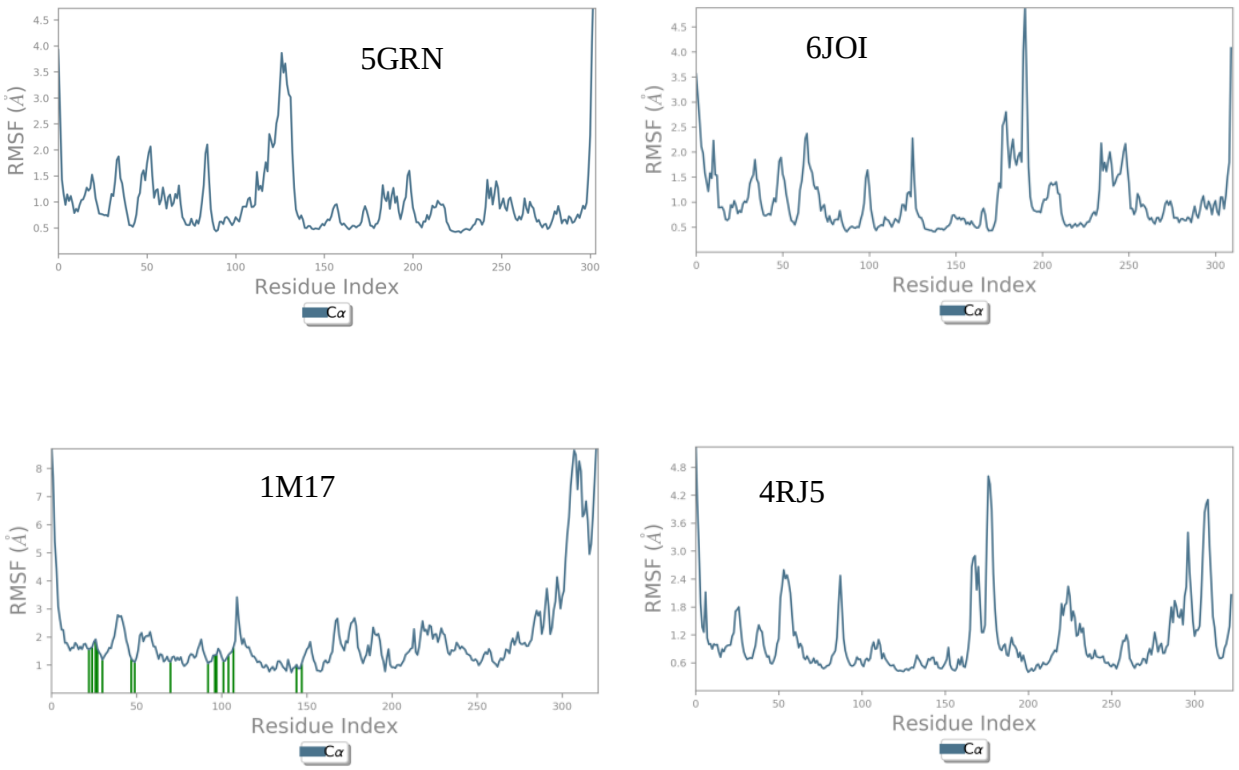


Figure S19. RMSF for the RTKs complexes with **AL-1**.

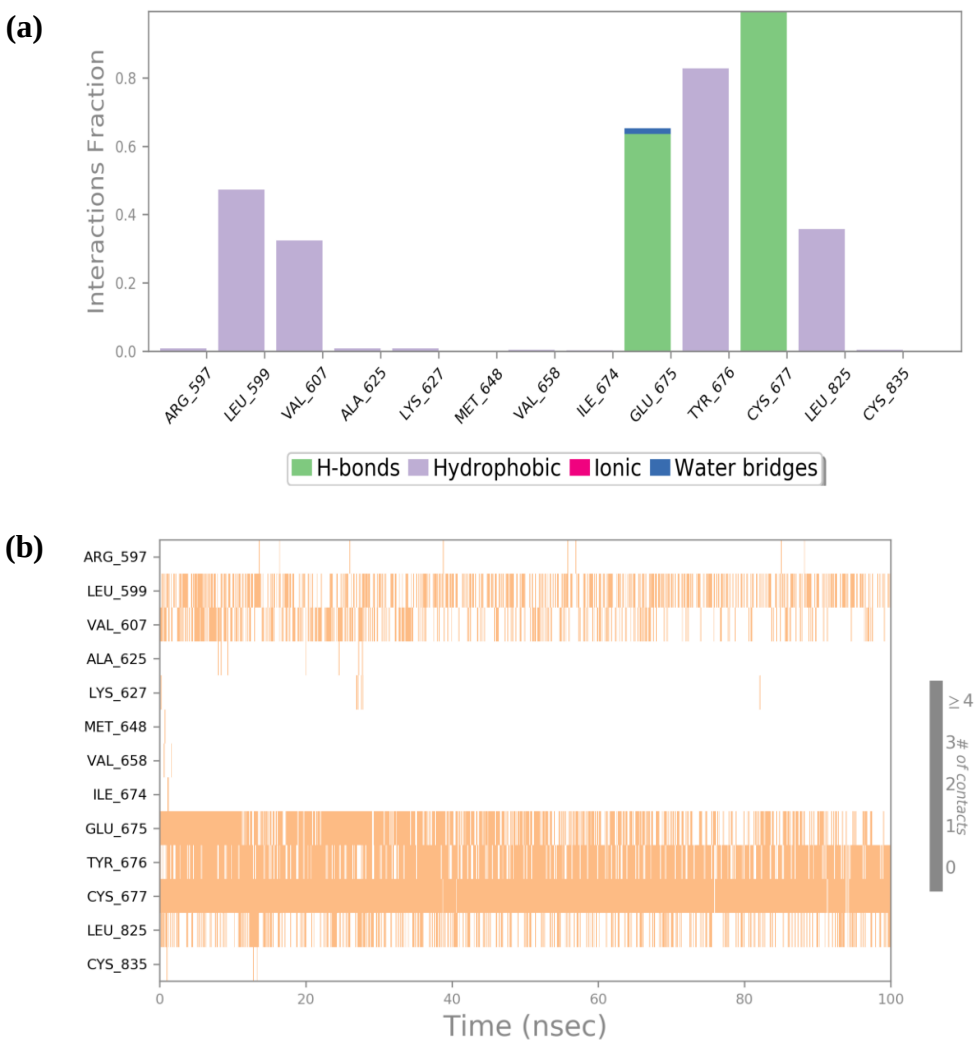


Figure S20. Analysis of the MD trajectories for the interactions (a) and total number of specific contacts (b) of RTK (6JOI) with the ligand **AL-1**.

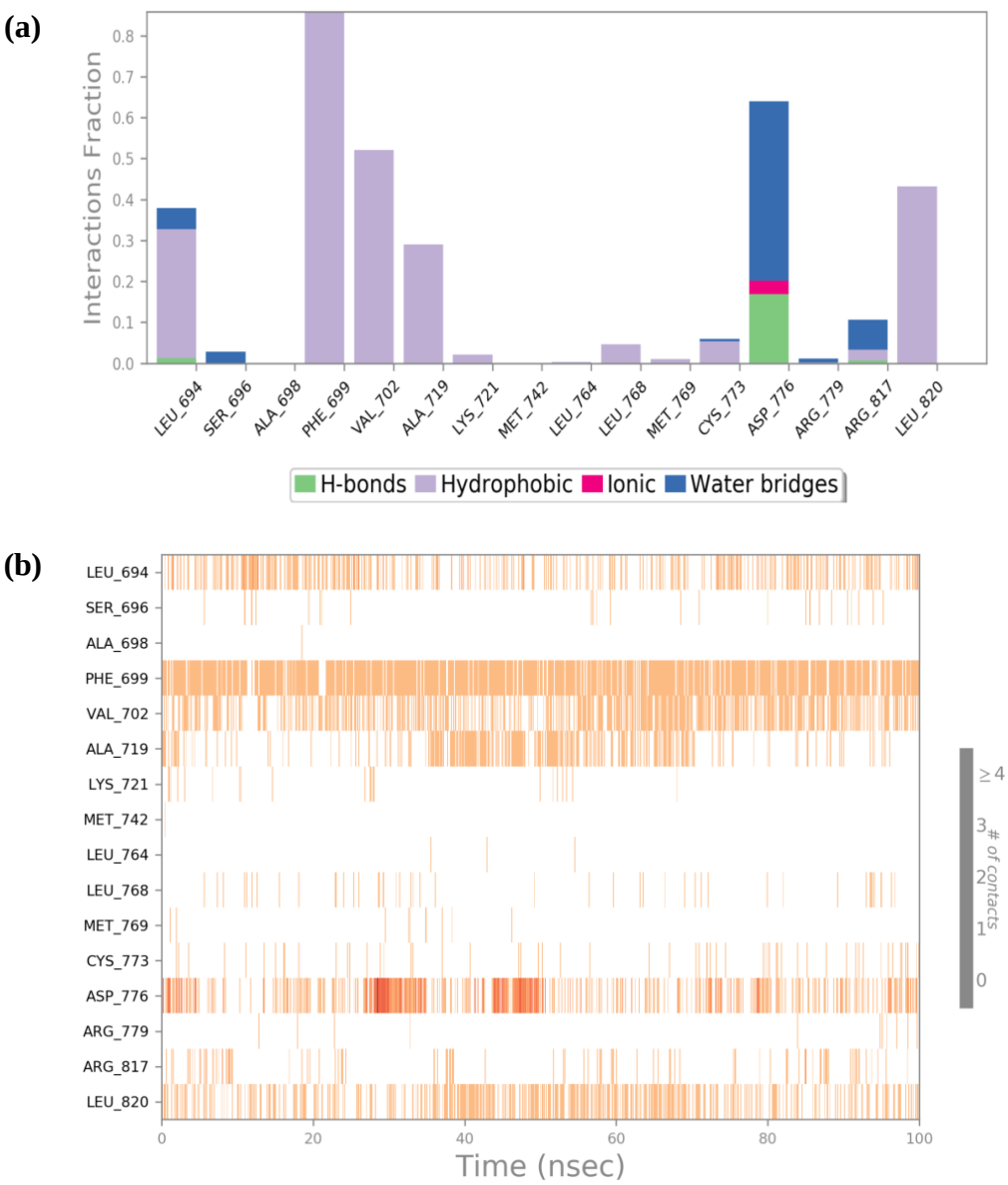


Figure S21. Analysis of the MD trajectories for the interactions (a) and total number of specific contacts (b) of RTK (1M17) with the ligand **AL-1**.

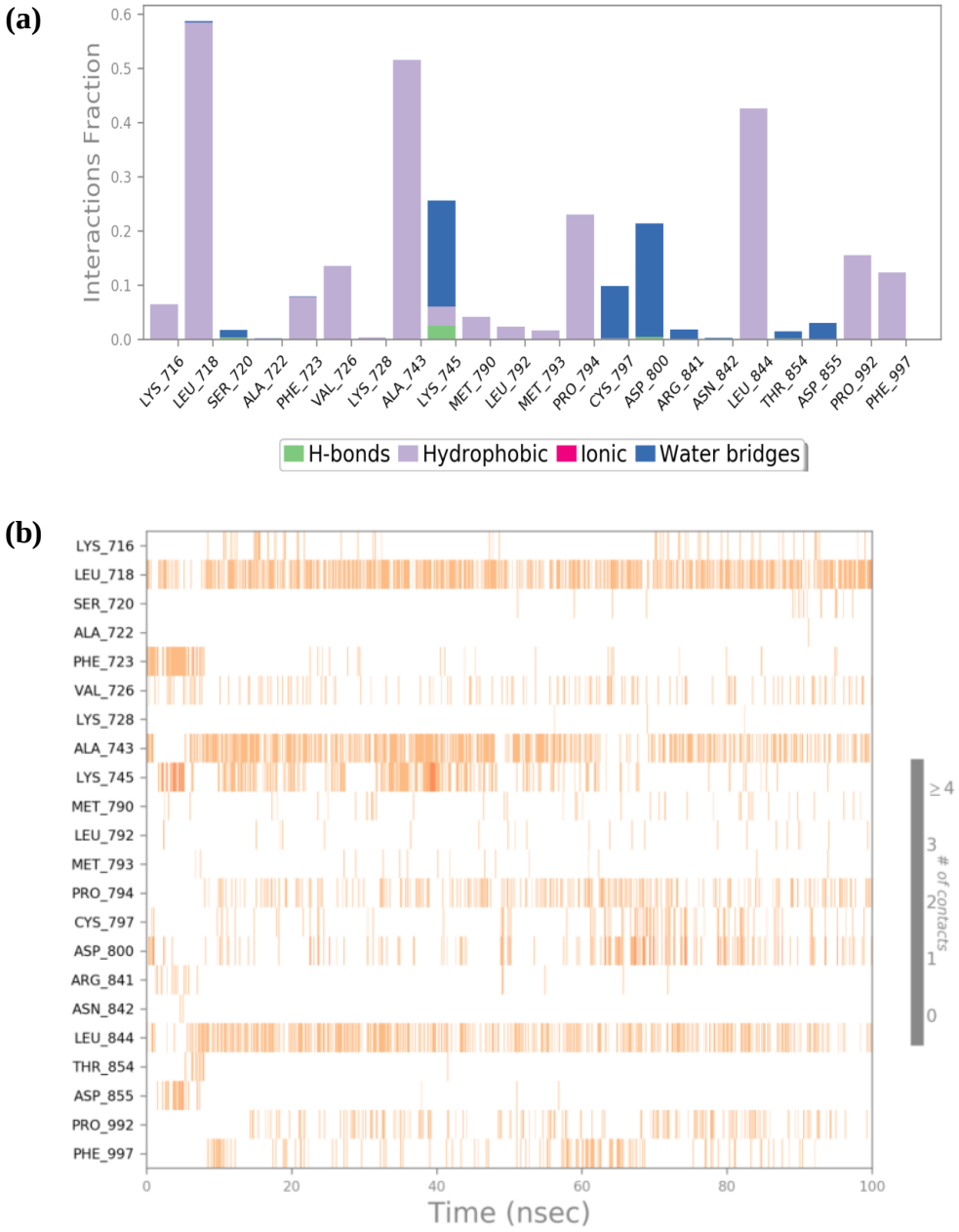


Figure S22. Analysis of the MD trajectories for the interactions (a) and total number of specific contacts (b) of RTK (4RJ5) with the ligand **AL-1**.

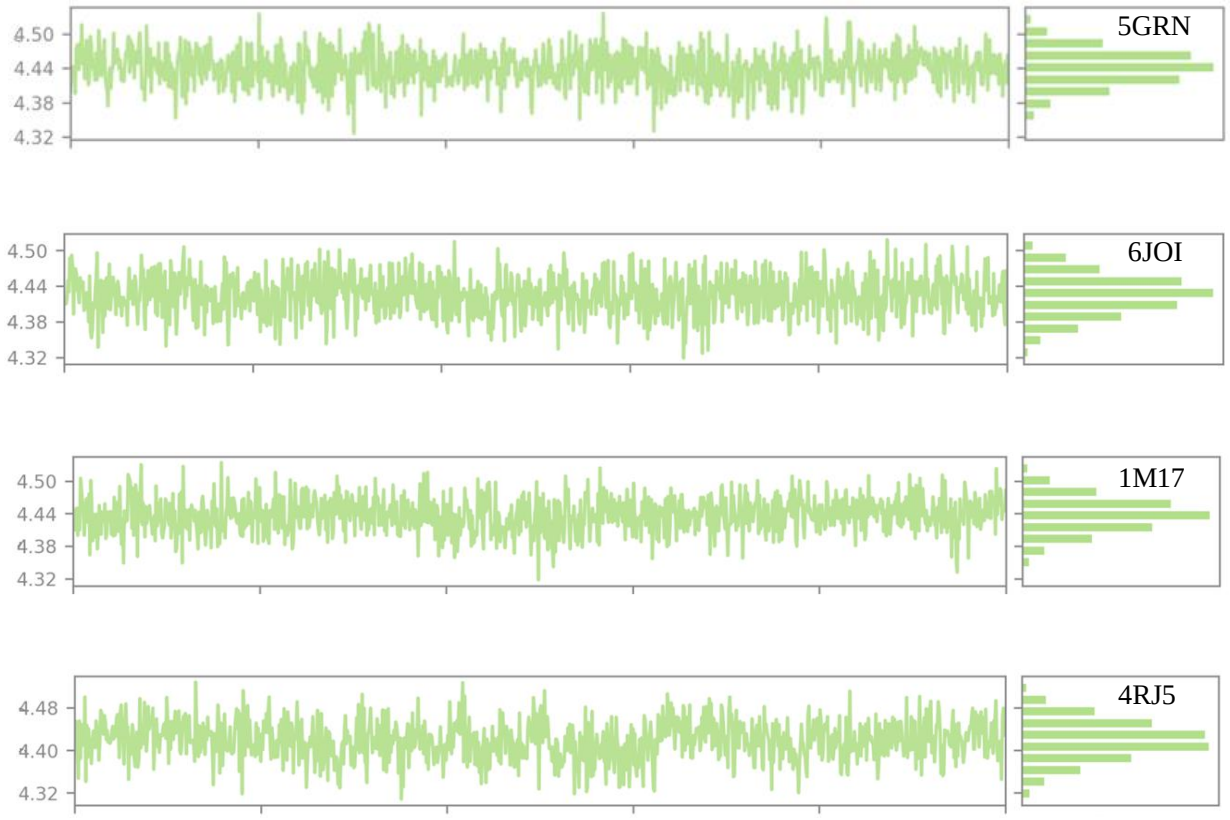


Figure S23. Radii of gyration for the RTKs complexes with **AL-1**.

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