Electronic Supplementary Material (ESI) for New Journal of Chemistry.

N,N',N''-Trisubstituted Guanidine Derivatives as DNA-Intercalators: Synthesis, Crystal Structures and Biophysical Investigations

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SUPPORTING INFORMATION

General methods

UV-Visible spectra were recorded in a Shimadzu UV-640 instrument. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 500 and 126 MHz spectrometer, respectively. DMSO-d_6 was used as solvent and tetramethylsilane (TMS) was used as an internal standard. High resolution mass spectra were recorded on a Thermo Exactive Orbitrap instrument.

X-ray crystallography

A Leica MZ 75 microscope was used to identify a suitable colourless block with very well-defined faces. The crystal mounted on a nylon loop was then placed in a cold nitrogen stream (Oxford) maintained at 110 K. A BRUKER Quest X-ray (fixed-Chi geometry) diffractometer with a PHOTON II detector was employed for crystal screening, unit cell determination and data collection. The goniometer was controlled using the APEX3 software suite.[1] The X-ray radiation employed was generated from a Mo-Iμs X-ray tube (K_α = 0.71073Å). Integrated intensity information for each reflection was obtained by reduction of the data frames with the program APEX3.[1] The integration method employed a three-dimensional profiling algorithm and all the data were corrected for Lorentz and polarization factors, as well as for crystal decay effects. Finally, the data were merged and scaled to produce a suitable data set. The absorption correction program SADABS[2] was employed to correct the data for absorption effects. Hydrogen atoms were placed in idealized positions and were set riding on the respective parent atoms. All the non-hydrogen atoms were refined with anisotropic thermal parameters. Absence of additional symmetry and voids were confirmed using PLATON (ADDSYM).[3] The structures were refined (weighted least squares refinement on F^2) to convergence.[4,5] Olex2 was employed for the final data presentation and structure plots.[4]

1. G. Murtaza , A. Badshah , M. Said , H. Khan , A. Khan , S. Khan , S. Siddiq , M. I. Choudhary , J. Boudreauc and F. G. Fontainec , *Dalton Trans*., **2011**, 40 , 9202 —9211
2. Murtaza, G.; Rauf, M. K.; Badshah, A.; Ebihara, M.; Said, M.; Gielen, M.; de Vos, D.; Dilshad, E.; Mirza, B. Synthesis, structural characterization and in vitro biological screening of some homoleptic copper(II) complexes with substituted guanidines. *Eur. J. Med. Chem.* **2012**, 48, 26– 35, DOI: 10.1016/j.ejmech.2011.11.029
3. Jeyalakshmi, K.; Selvakumaran, N.; Bhuvanesh, N. S. P.; Sreekanth, A.; Karvembu, R. DNA/protein binding and cytotoxicity studies of copper(II) complexes containing N,

N′, N″-trisubstituted guanidine ligands. *RSC Adv*. **2014**, 4, 17179–17195, DOI: 10.1039/c4ra01459f

4. Jeyalakshmi, K.; Arun, Y.; Bhuvanesh, N. S. P.; Perumal, P. T.; Sreekanth, A.; Karvembu, R. DNA/protein binding, DNA cleavage, cytotoxicity, superoxide radical scavenging and molecular docking studies of copper(II) complexes containing N-benzyl-N′-aryl-N″-benzoylguanidine ligands. *Inorg. Chem. Front*. **2015**, 2, 780– 798, DOI: 10.1039/C4QI00234B
5. Jeyalakshmi, K.; Haribabu, J.; Balachandran, C.; Swaminathan, S.; Bhuvanesh, N. S. P.; Karvembu, R. Coordination Behavior of N,N′,N″-Trisubstituted Guanidine Ligands in Their Ru–Arene Complexes: Synthetic, DNA/Protein Binding, and Cytotoxic Studies. *Organometallics* **2019**, 38, 753– 770, DOI: 10.1021/acs.organomet.8b00702

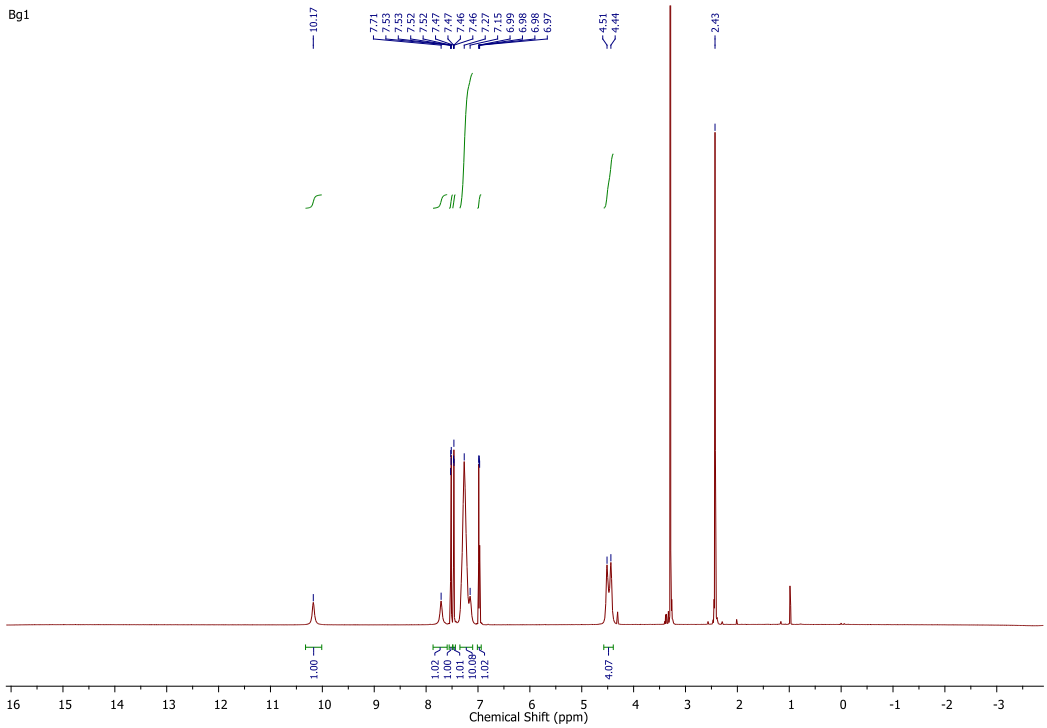

Fig. S1. ^{1}H NMR spectrum of **L1**

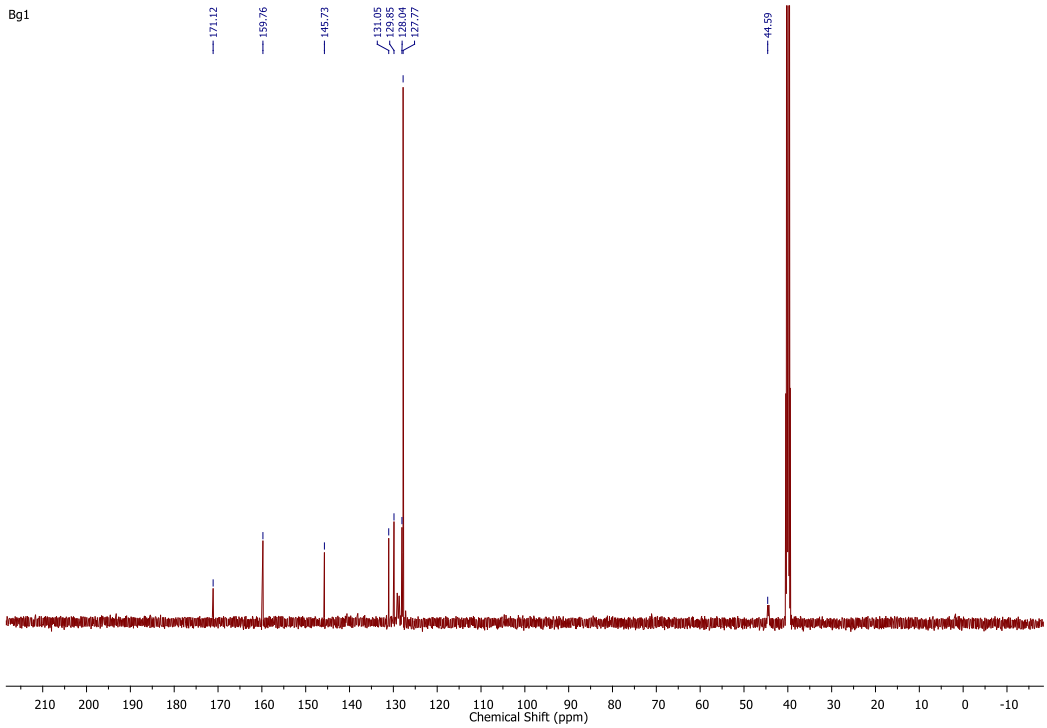

Fig. S2. ^{13}C NMR spectrum of **L1**

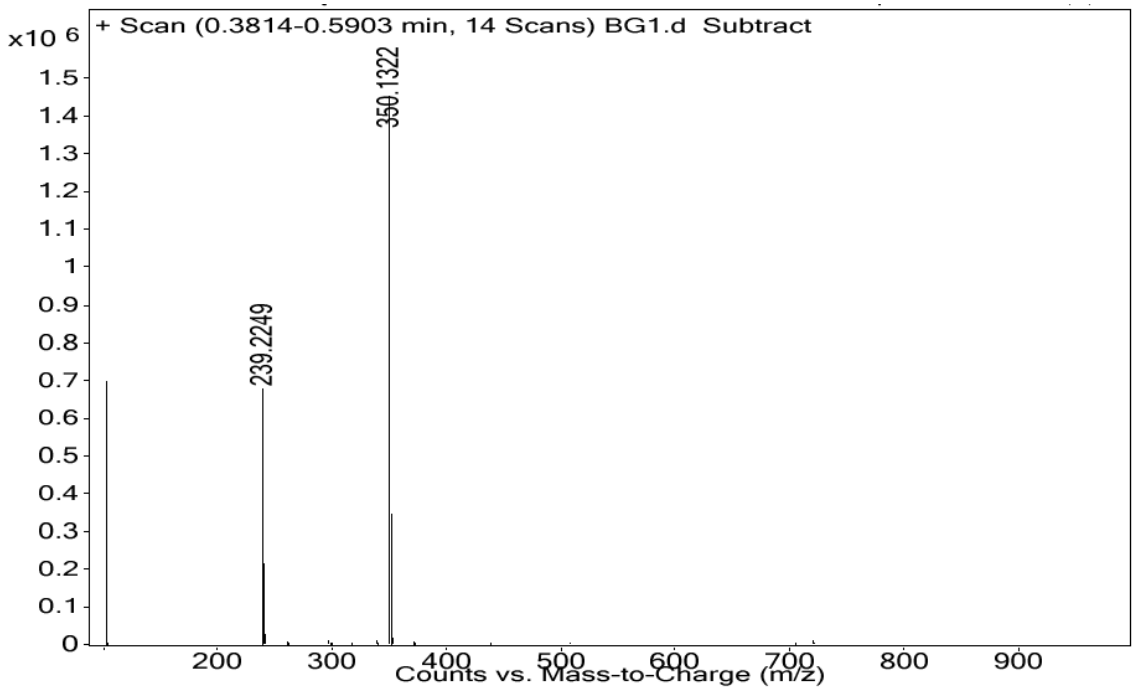

Fig. S3. Mass spectrum of **L1**

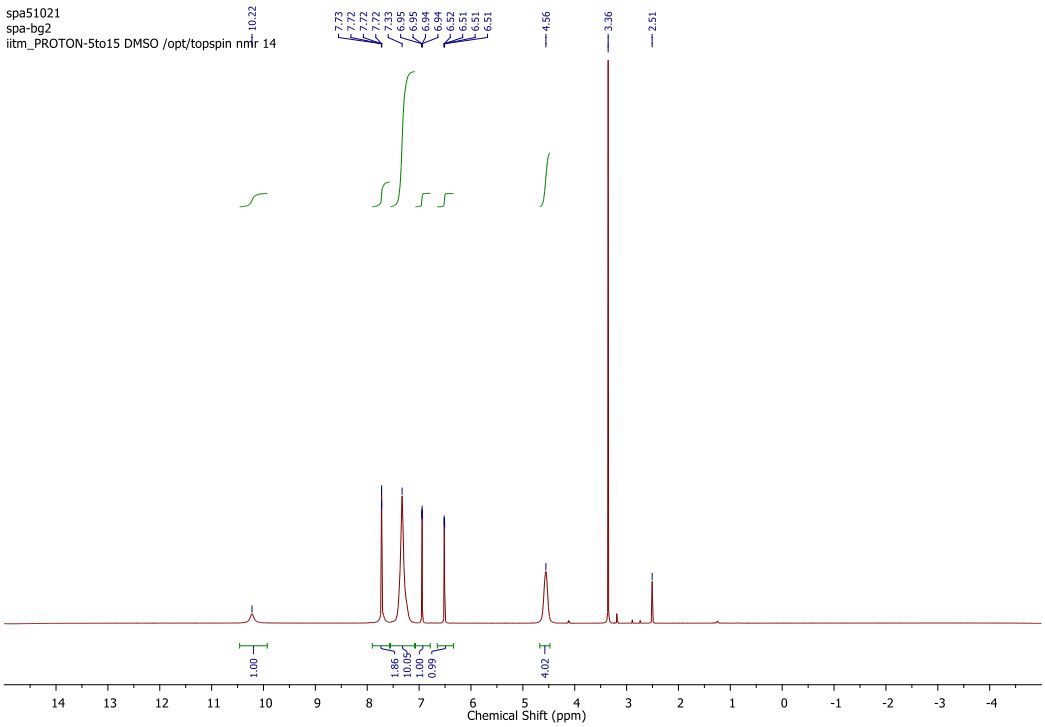

Fig. S4. ^{1}H NMR spectrum of **L2**

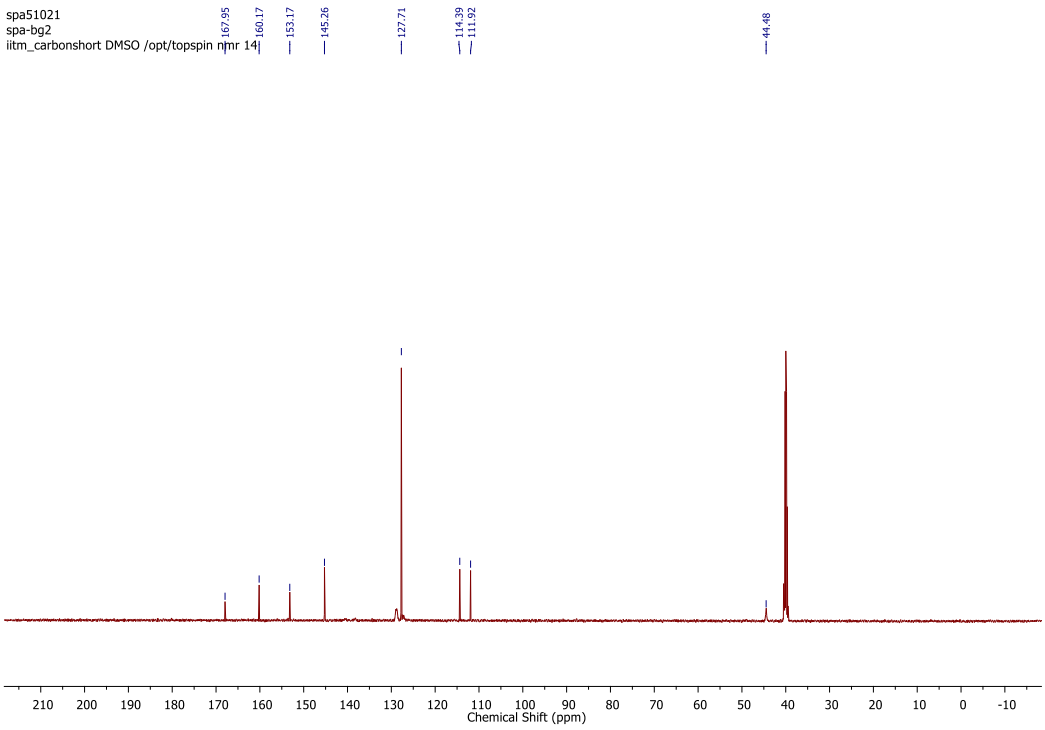

Fig. S5. ^{13}C NMR spectrum of **L2**

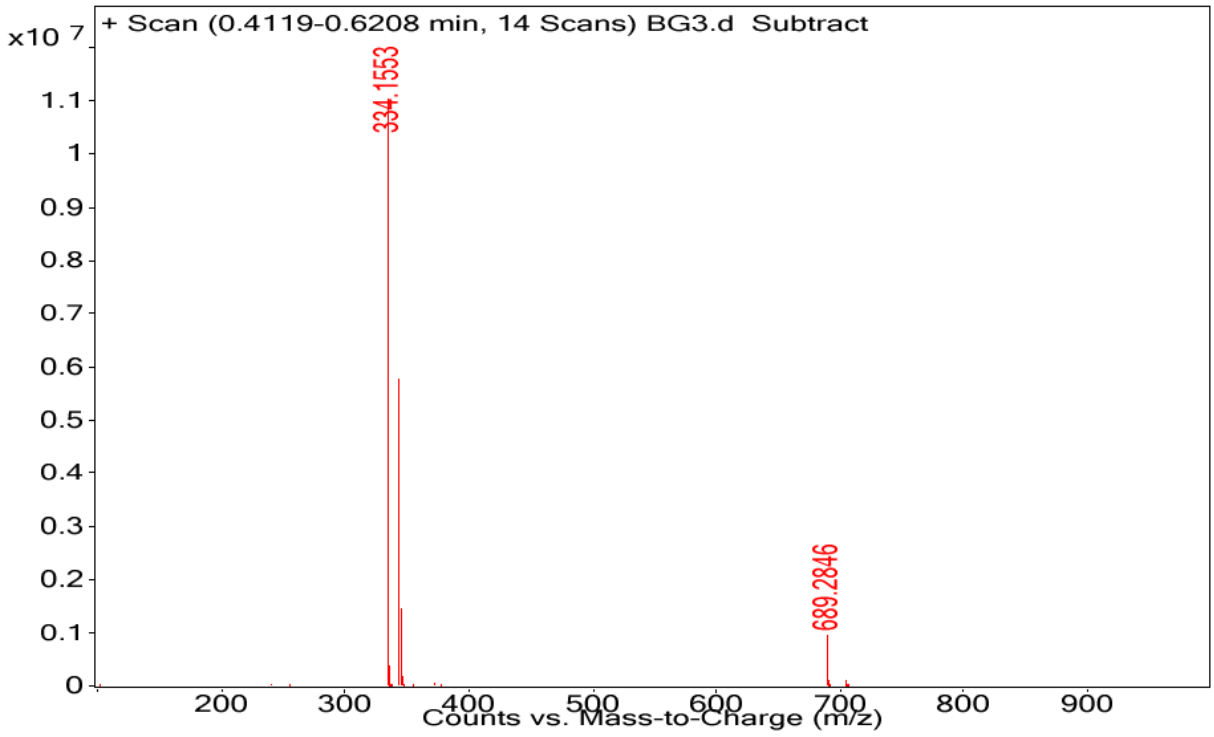

Fig. S6. Mass spectrum of **L2**

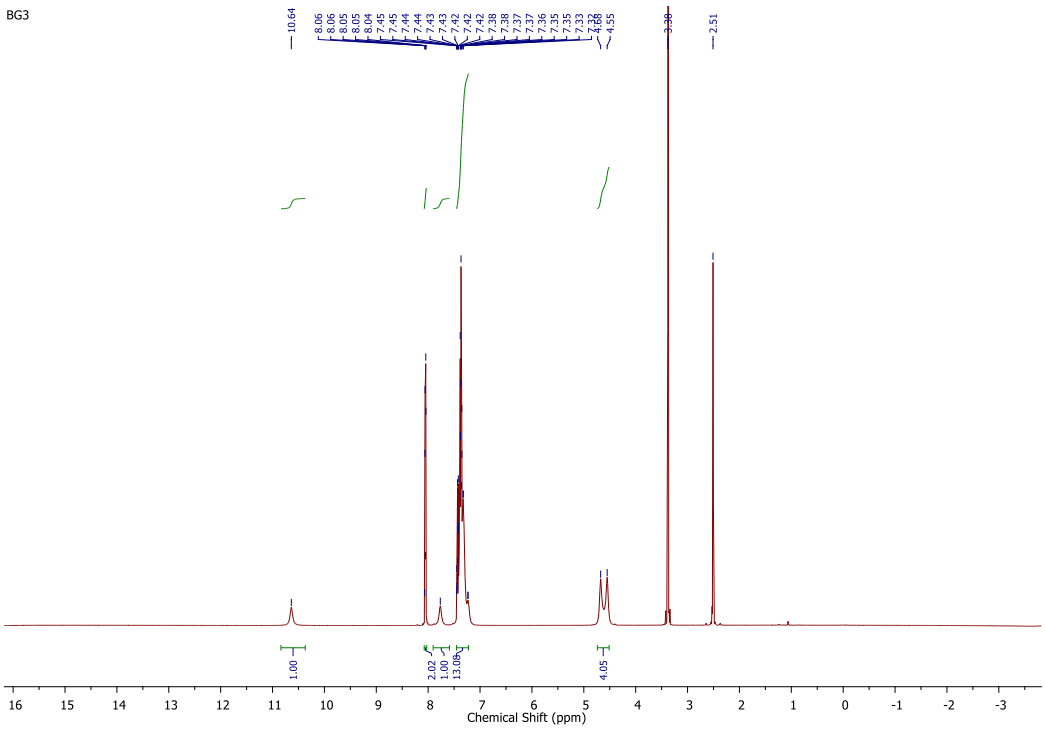

Fig. S7. ^{1}H NMR spectrum of **L3**

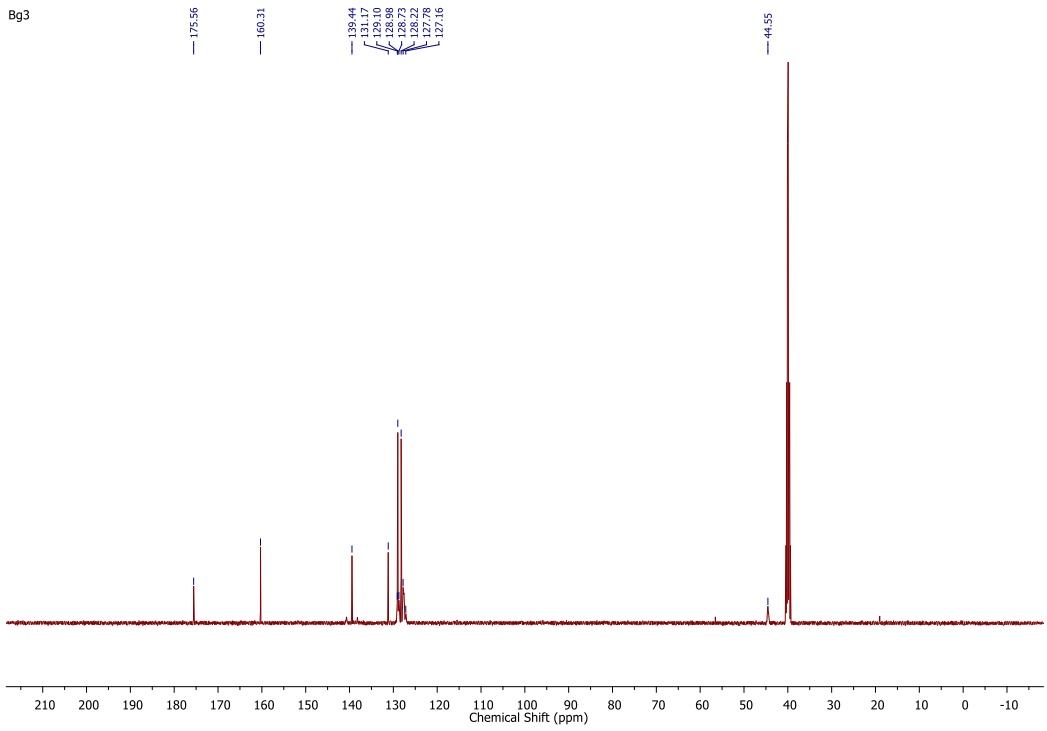

Fig. S8. ^{13}C NMR spectrum of **L3**

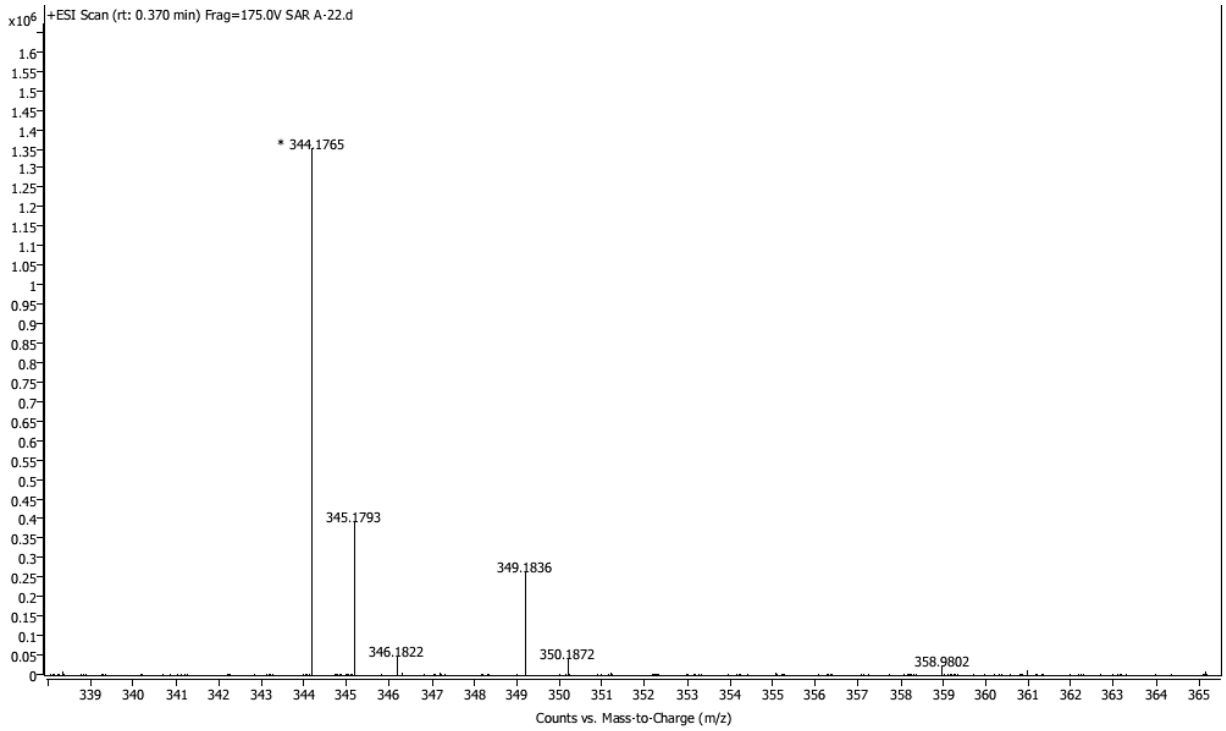

Fig. S9. Mass spectrum of **L3**

Table S1. Crystal data and refinement parameters for **L1-L3**

	L1	**L2**	**L3**
Empirical formula	$C_{20}H_{19}N_3OS$	$C_{20}H_{19}N_3O_2$	$C_{22}H_{21}N_3O$
Formula weight	349.44	333.38	343.42
Temperature	110.0 K	110.0 K	110.0 K
Wavelength	0.71073 Å	0.71073 Å	0.71073 Å
Crystal system	Orthorhombic	Triclinic	Orthorhombic
Space group	*Pbca*	*P-1*	*Pbca*
Unit cell dimensions	13.2629(10) Å	9.3435(5) Å	15.0645(7) Å
a	12.0772(9) Å	12.5993(7) Å	11.7756(5) Å
b	22.1882(16) Å	15.2448(9) Å	20.4088(9) Å
c	90°	84.540(2)°	90°
α	90°	72.858(2)°	90°
β	90°	81.087(2)°	90°
γ			
Volume	3554.1(5) $Å^3$	1691.88(17) $Å^3$	3620.4(3) $Å^3$
Z	8	4	8
Density (calculated)	1.306 Mg/m^3	1.309 Mg/m^3	1.260 Mg/m^3
Absorption coefficient	0.195 mm^{-1}	0.086 mm^{-1}	0.079 mm^{-1}
F(000)	1472	704	1456
Crystal size	0.547 × 0.492 × 0.458 mm^3	0.208 × 0.186 × 0.038 mm^3	0.407 × 0.326 × 0.216 mm^3
Theta range for data collection	2.393 to 24.998°	2.098 to 24.998°	2.411 to 27.483°
Index ranges	-15<=h<=15, -14<=k<=13, -23<=l<=26	-11<=h<=11, -14<=k<=14, -18<=l<=18	-19<=h<=19, -15<=k<=15, -26<=l<=26
Reflections collected	27648	34170	30883
Independent reflections	3127 [R(int)=0.0432]	5950 [R(int)= 0.0397]	4131 [R(int) = 0.0477]
Completeness to theta =	24.998° 100.0 %	24.998° 99.9 %	25.242° 99.8 %
Absorption correction	Semi-empirical from equivalents	Semi-empirical from equivalents	Semi-empirical from equivalents
Max. and min. transmission	0.4305 and 0.3809	0.7456 and 0.6997	0.4305 and 0.3944

Data / restraints / parameters	3127 / 0 / 226	5950 / 0 / 451	4131 / 0 / 235
Goodness-of-fit on F^2	1.036	1.064	1.035
Final R indices [I>2sigma(I)]	R1 = 0.0328, wR2 = 0.0773	R1 = 0.0362, wR2 = 0.0818	R1 = 0.0399, wR2 = 0.0914
R indices (all data)	R1 = 0.0412, wR2 = 0.0830	R1 = 0.0486, wR2 = 0.0921	R1 = 0.0542, wR2 = 0.0987
Largest diff. peak and hole	0.308 and -0.257 e.Å^{-3}	0.219 and -0.265 e.Å^{-3}	0.171 and -0.197 e.Å^{-3}
CCDC No.	2218225	2218226	2218227

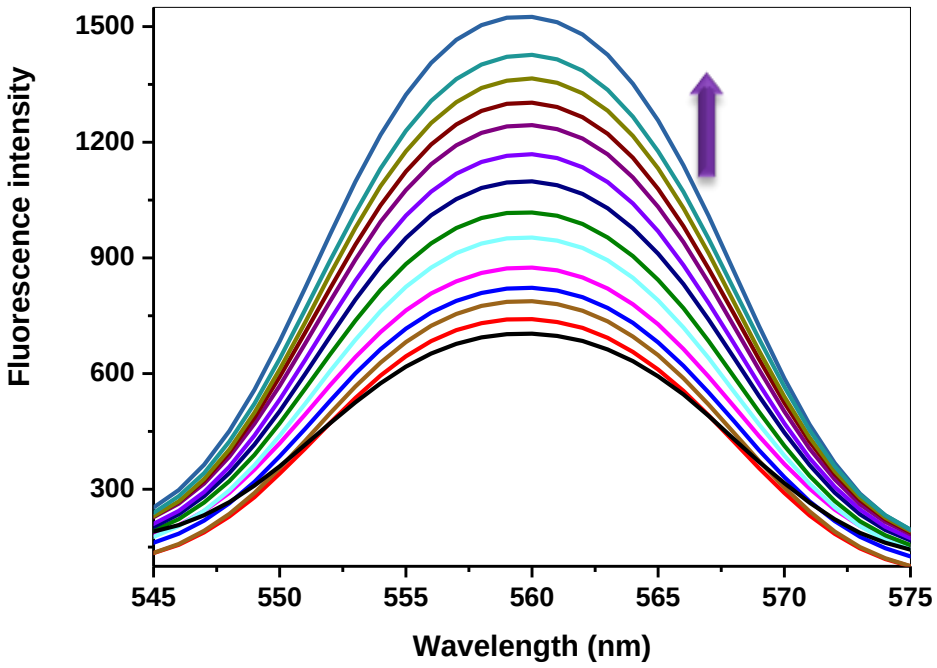

Fig. S10. Fluorescence spectra of **L2** (50 µM) with incremental addition of CT-DNA (0-6 µM) in Tris-HCl buffer of pH 7.4 at 308 K.

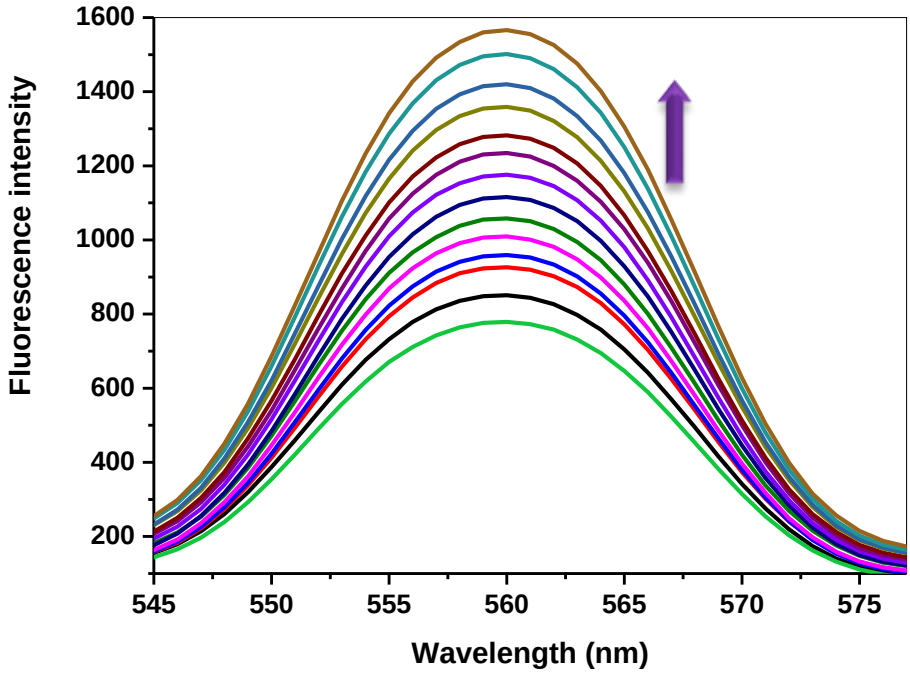

Fig. S11. Fluorescence spectra of **L2** (50 µM) with incremental addition of CT-DNA (0-6 µM) in Tris-HCl buffer of pH 7.4 at 318 K.

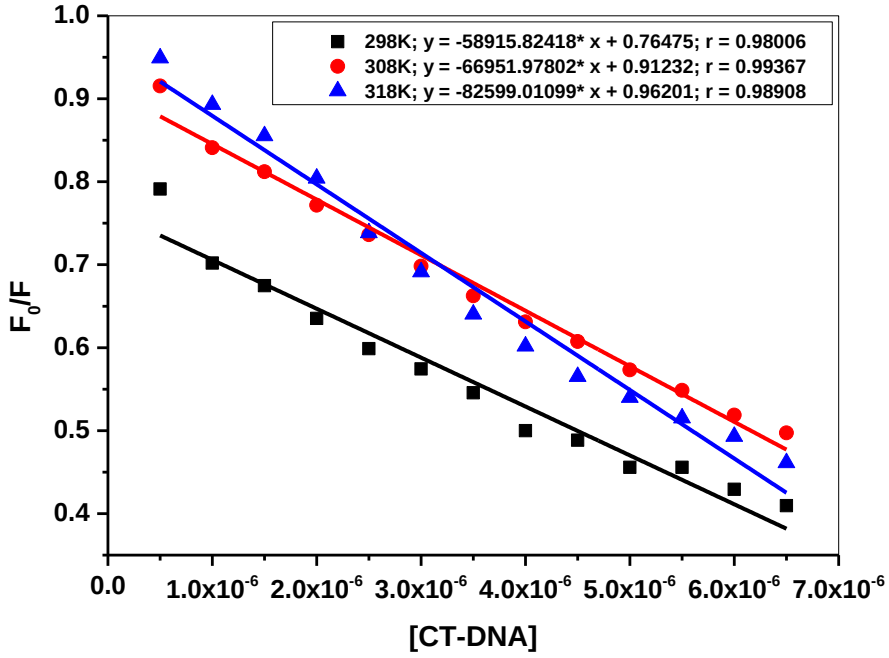

Fig. S12. Plot of F_0/F versus [CT-DNA] for **L2** by CT-DNA at three different temperatures.

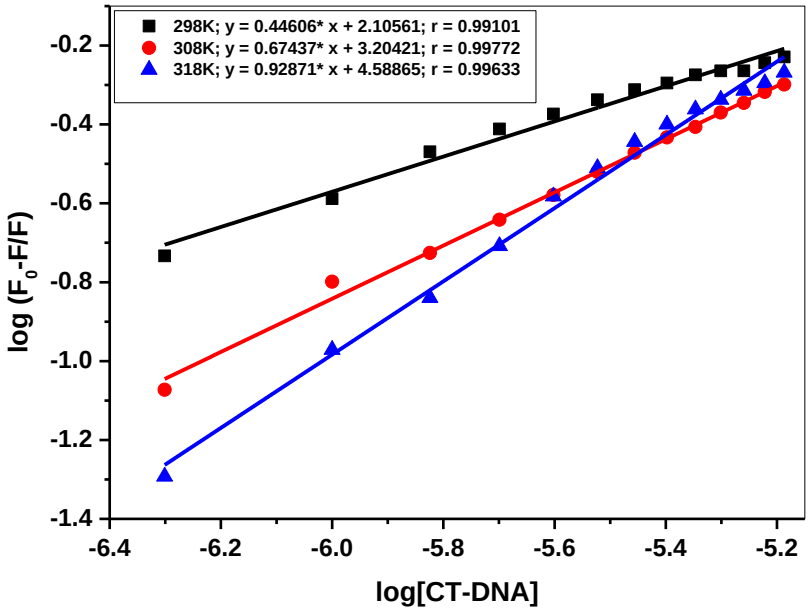

Fig. S13. Plot of log [F-F_0/F] vs. log [CT-DNA] of **L2** at three different temperatures.

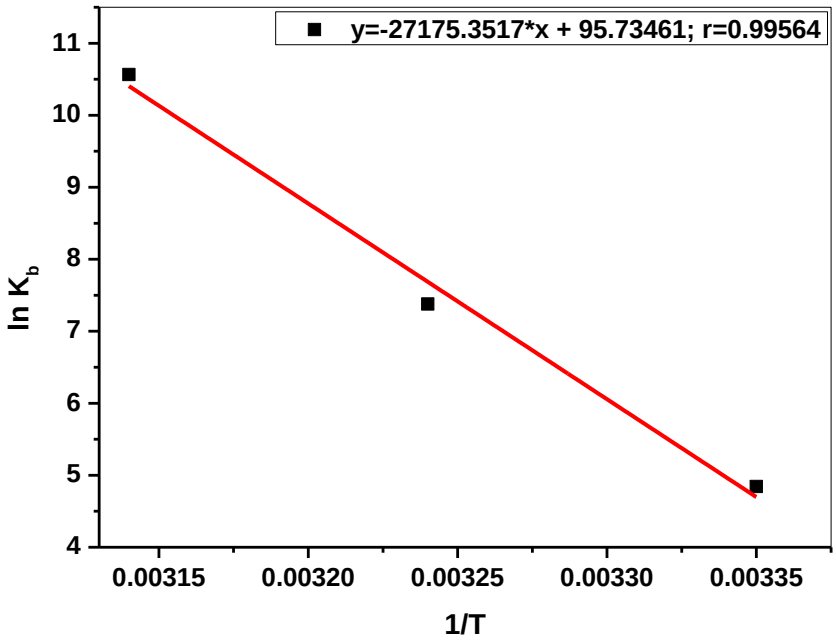

Fig. S14. van't Hoff plot of **L2** with CT-DNA.

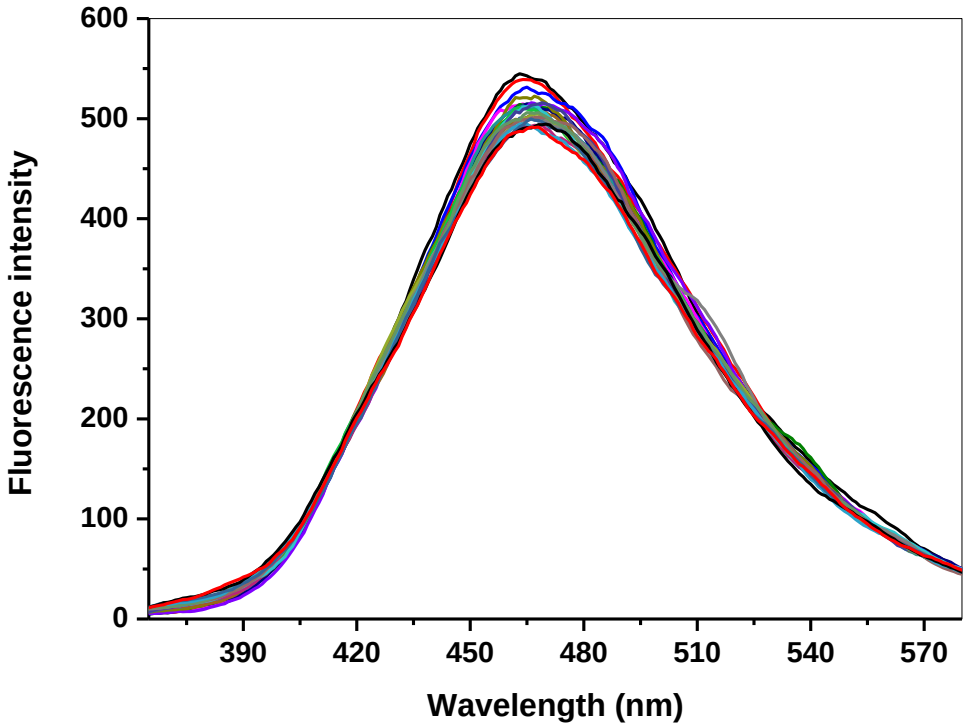

Fig. S15. Fluorescence changes for the titration of **L2** (0-30 µM) with CT-DNA-Hoechst 33258 system

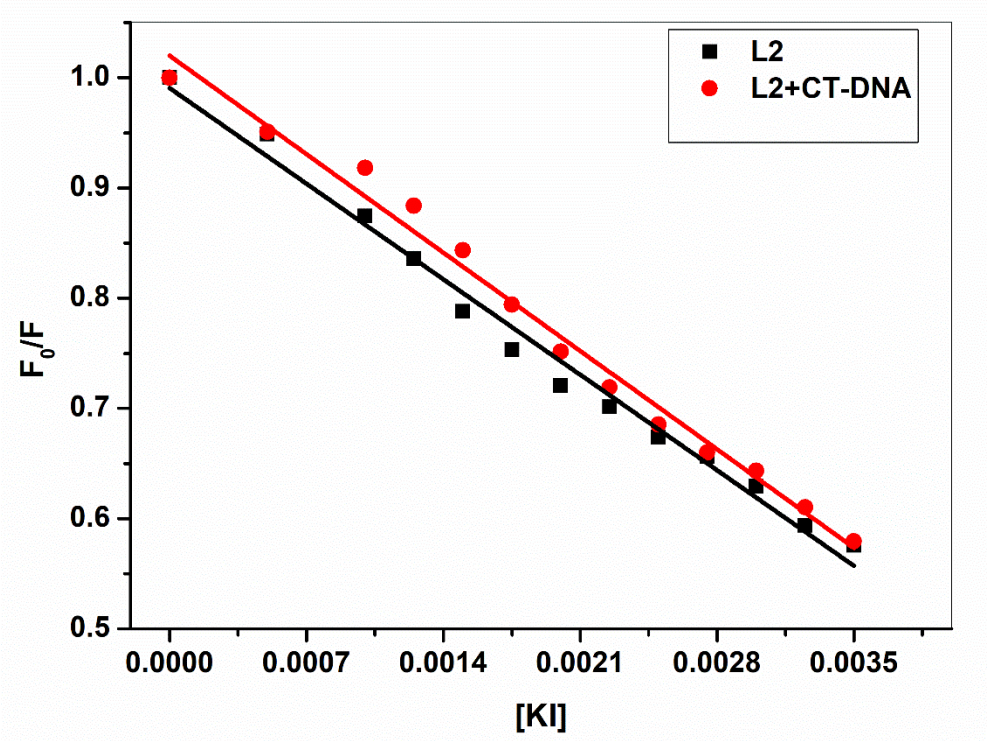

Fig. S16. Fluorescence quenching plot of **L2** by KI
in the absence and presence of CT-DNA

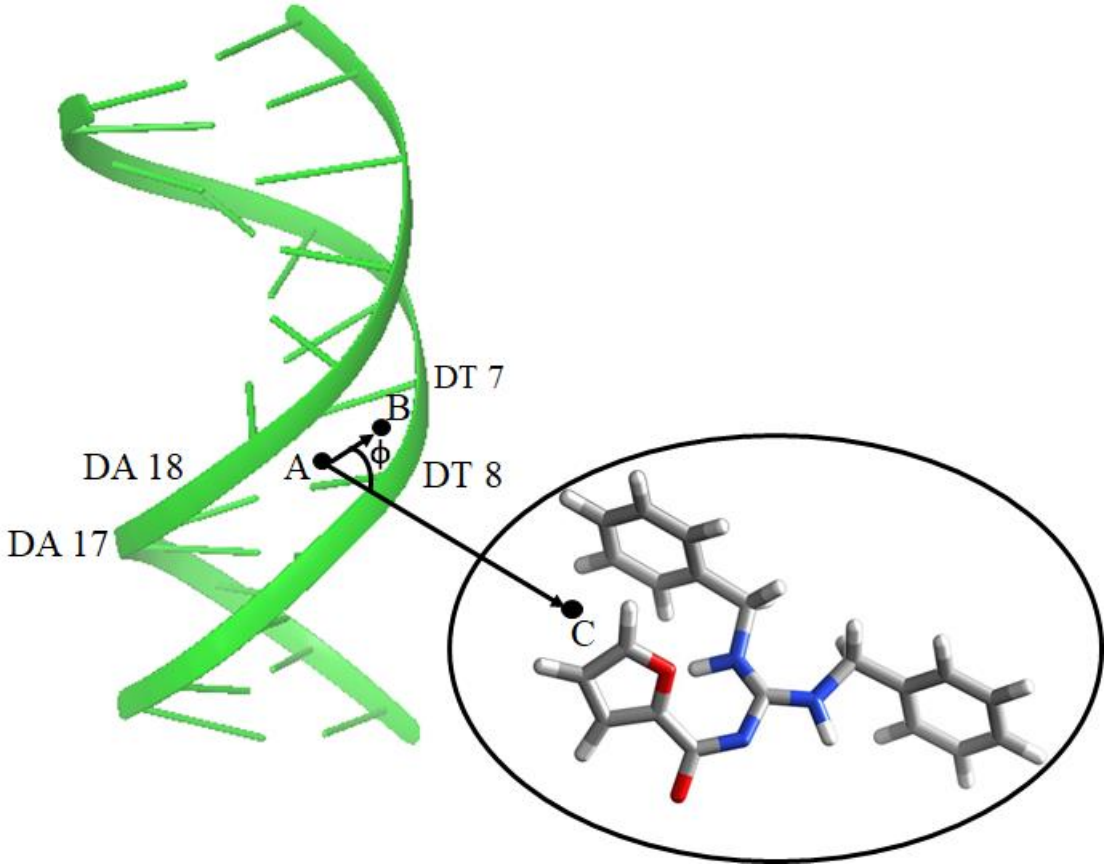

Fig. S17. Two-dimensional view of the vectors chosen for collective variables. Point A is the COM of four bases labeled (DT7, DT8, DA17 and DA18); B is the COM of the sugar groups of DT7 and DA18. Point C is the COM of the drug L2. AB is the body fixed vector and AC is the distance variable. The angle between AB and AC is the angle variable.

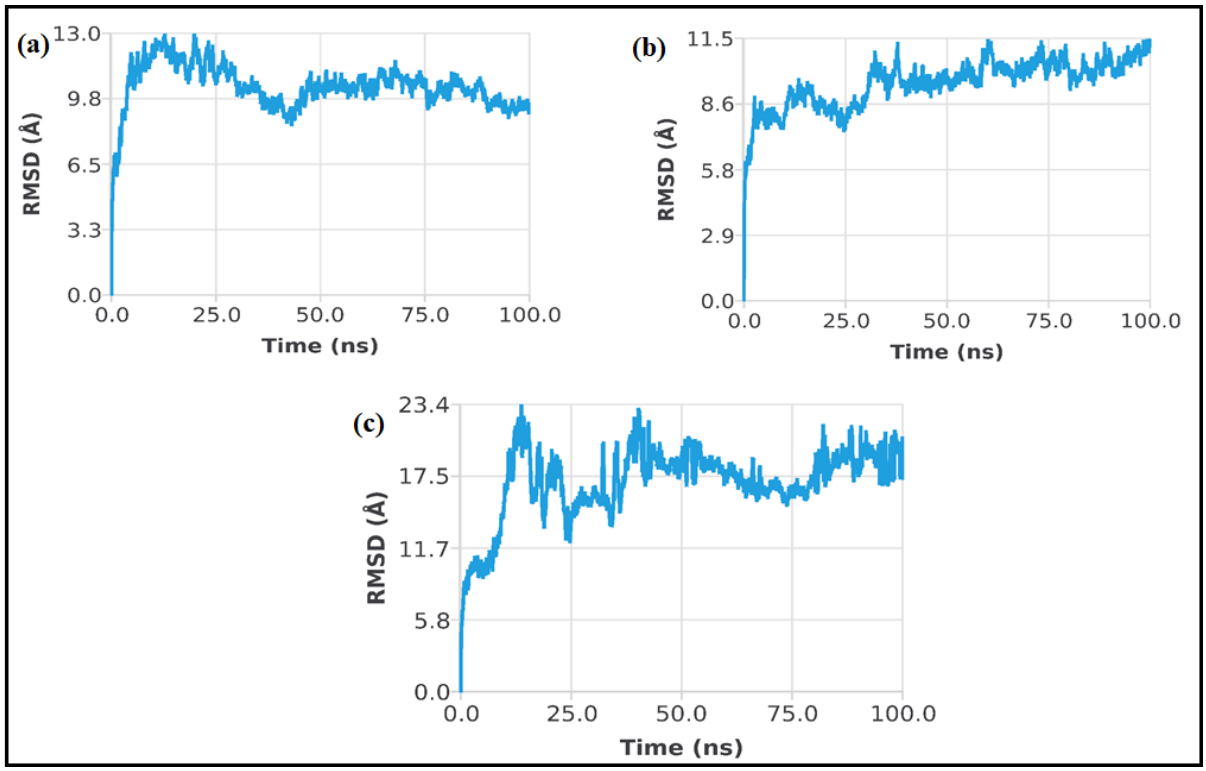

Fig. S18. RMSD graphs for **L1** (a), **L2** (b) and **L3** (c)

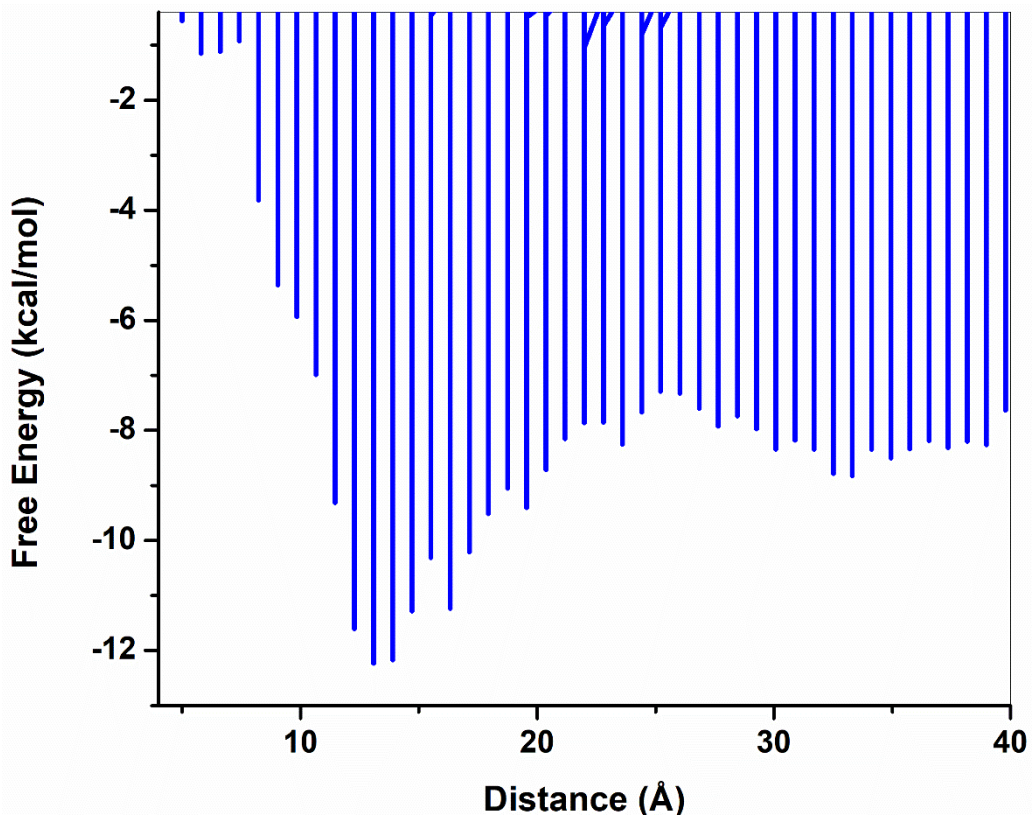

Fig. S19. 1D free energy profile of L1 ligand.

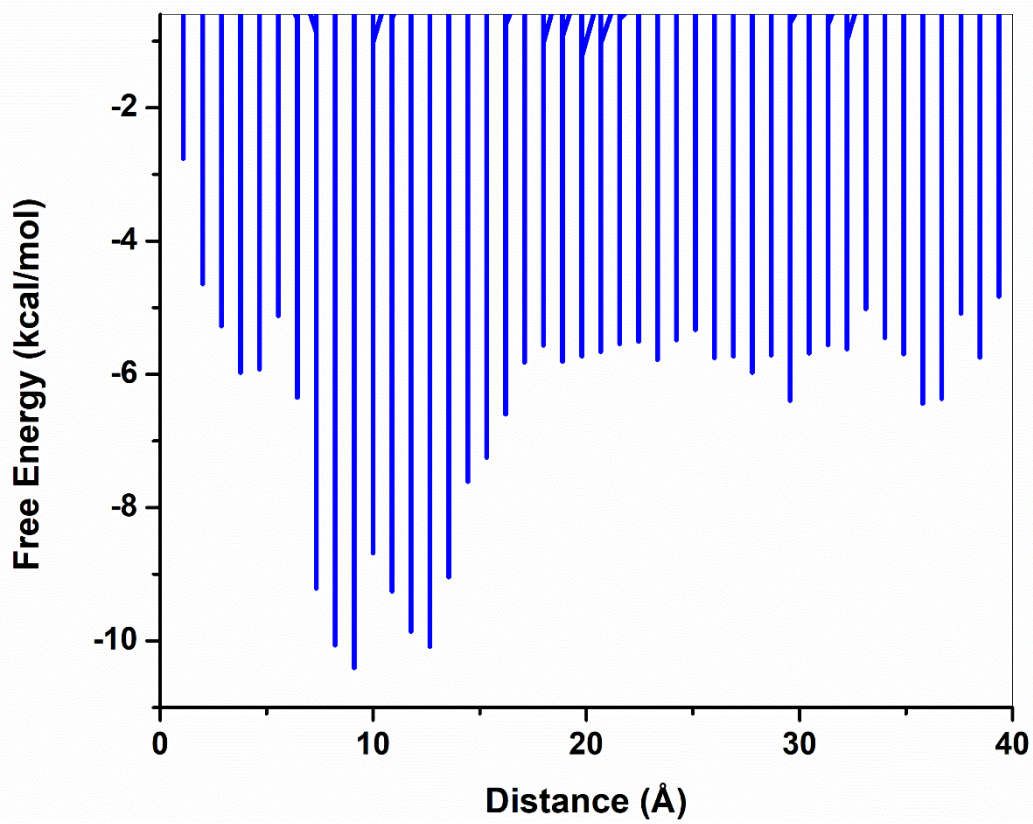

Fig. S20. 1D Free energy profile of L2 ligand.

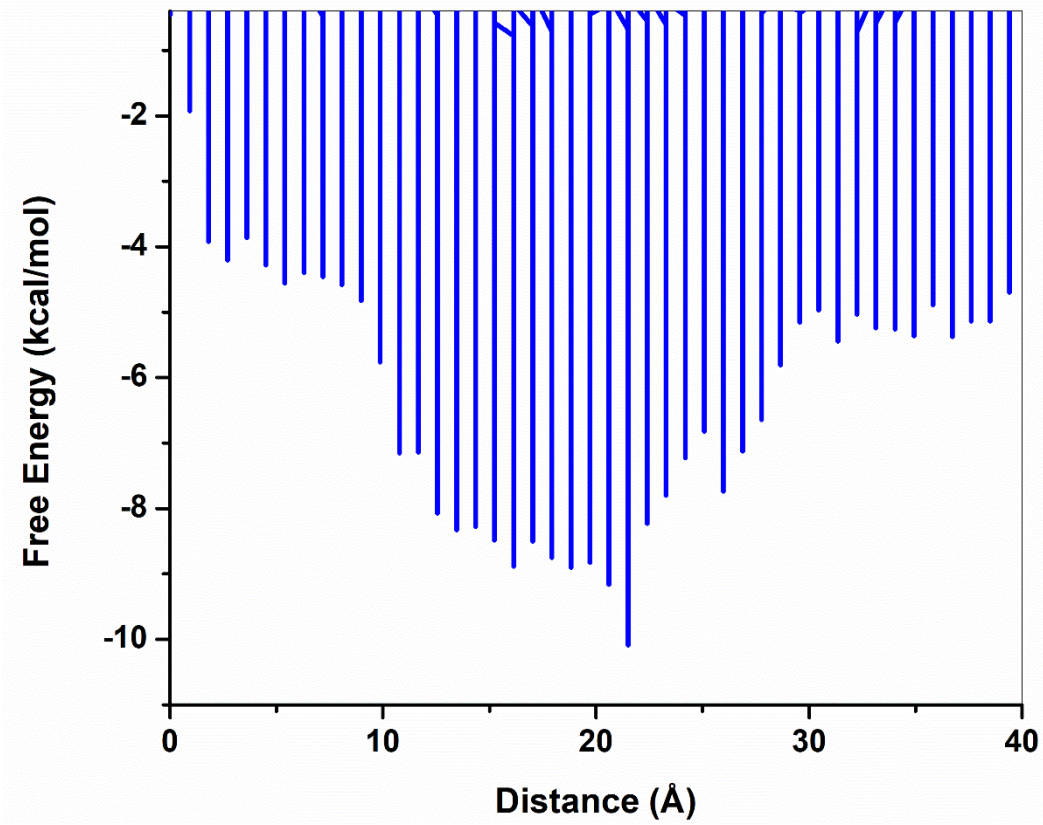

Fig. S21. 1D Free energy profile of L3 ligand.

Table S2. Type of interactions present in the free energy minimum of L1 ligand

S.No	Frame number	Type of interactions	Interacting residues	Collective variables (Distance (Å), Angle (⁰))	Energy kcal/mol	Rise DC11 DG14	Roll DC11 DG14	Twist DC11 DG14
1	208	π- π (1), π-cation (2), water mediated hydrogen bonding (1)	DA17, DC13,	12.36, 96.90	-11.50	2.51 10.8	94.13 11.61	57.17 -28.54
2	403	Unbound	-	14.34, 103.57	-10.70	-6.24 12.78	-24.58 -14.94	-91.39 -39.39
3	73	Unbound	-	15.08, 100.65	-11.28	8.81 11.75	-11.75 -18.88	31.39 -44.77
4	849	π- π (1), water mediated hydrogen bond (1)	DC-11	14.27, 98.32	-11.98	-12.5 12.55	-6.93 -43.86	-52.26 -51.78
5	620	Water mediated hydrogen bond (1)	DC-9	13.77,102.40	-12.17	-6.98 15.04	-15.29 -77.75	-99.86 41.39
6	863	Unbound	-	13.67,102.99	-11.31	-6.2 -12.83	-29.76 -60.69	-121.97 -43.8
7	860	π- cation (1), water mediated hydrogen bond (2)	DC-11	13.14,101.53	-11.74	-10.08 11.67	-6.41 -68.55	-89.8 -52.24
8	442	Unbound	-	12.82,102.99	-10.58	-7.6 6.99	-3.05 -73.13	-108.5 -9.34
9	889	Unbound	-	12.25,99.49	-10.81	-7.5 11.22	-28.63 -18.08	-98.86 -22.83
10	890	Unbound	-	12.75,94.23	-12.02	-7.36 9.35	-25.65 -44.64	-85.28 -68.27
11	864	Unbound	-	13.24,96.28	-12.23	-9.03 10.15	-12.61 -52	-112.68 -62.82
13	859	Unbound	-	13.38,93.07	-12.02	-8.55 10.53	-18.71 -47.99	-86.58 61.49
14	172	Unbound	-	13.74,93.36	-11.18	2.33	89.08	122.4

						12.12	-17.47	-62.85
15	862	Unbound	-	13.74,90.44	-10.48	-8 11.86	-1.61 -30.11	-116.42 -61.65
16	876	Unbound	-	12.60,91.02	-9.61	-6.05 13.69	-11.74 -38.56	-67.73 -29.1
17	885	Hydrogen bonds (2)	DC-9, DC-11	12.43,90.15	-9.61	-6.84 10.95	14.83 1.99	-70.75 -45.18
18	861	π- π (2), π-cation (2), Water mediated hydrogen bond (1)	DC-11, DG-10	12.39,91.61	-9.61	-7.52 12.67	-38.32 -53.05	-89.86 -57.82
19	879	Unbound	-	12.32,91.90	-10.90	-6.96 12.62	1.18 -20.89	-88.9 -4.32
20	888	Unbound	-	12.04,94.53	-10.90	-5.69 11.27	-7.02 -18.21	-106.11 -43.15
21	829	π- π (1), π-cation (1)	DC-9	11.86,100.36	-9.31	-10.03 7.93	-1.07 -15.41	-62.27 -53.69
22	880	Unbound	-	11.61,99.49	-9.31	-6.51 13.52	-17.14 -77.42	-95.58 -52.62
23	621	Unbound	-	12.29,106.49	-8.88	-7.5 12	-10.15 -29.81	-68.83 -58.07
24	848	π- π (1), Hydrogen bond (1), Water mediated hydrogen bond (2)	DC-11, DG-10	13.56,108.53	-10.21	-9.5 11.63	-34.85 -33.68	-81.92 -44.71
25	71	π- cation (1), Water mediated hydrogen bond (1)	DG-10	14.23,109.70	-10.21	5.49 10.45	-38.51 -37.05	57.87 -52.41
26	457	π- π (1), π-cation (1)	DA-18	14.91,106.20	-10.70	-9.92 14.01	-6.31 -37.98	-83.72 -37.09
27	858	Unbound	-	15.12,106.20	-9.83	-10.61 5.43	-30.47 -5.29	-74.24 -93.71
28	852	π- π (1)	DC-11	15.55,100.95	-10.31	-13.49	12.64	-66.32

						10.17	-38.11	-66.05
29	851	π- π (1), Water mediated hydrogen bond (1)	DG-10	16.40,104.16	-10.31	-13.34 11.73	-10.72 -45.52	-61.98 -25.34
30	846	Unbound	-	16.89,103.86	-8.20	-9.77 18.25	-22.68 -36.76	-66.1 -36.91
31	854	Unbound	-	18.06,95.11	-9.51	-9.53 10.15	-12.49 -43.18	-87.92 -71.4
32	169	Unbound	-	16.93,97.74	-10.21	1.62 14.93	66.52 -52.85	75.88 -18.17
33	853	pi-cation (2)	DC-11	16.43,97.15	-10.91	-9.51 11.83	-26.24 -27.87	-84.44 -31.63
34	857	π- π (2), π-cation (2), Water mediated hydrogen bond (1)	DG-10, DC-11	16.43,99.78	-11.23	-12.34 11.05	-21.56 -27.03	-59.53 -42.59
36	623	π- π (1), Hydrogen bond (1)	DC-9	15.94,91.02	-10.05	-8.08 13.8	-30.95 -25.63	-79.51 -5.08
37	855	Unbound	-	15.55,92.19	-9.55	-11.1 5.97	-17.7 -6.13	-5023 -89.9
38	171	Unbound	-	15.47,90.15	-8.94	3.74 12.5	81.04 -3.14	128.94 -27.29
39	850	π- cation (1), Water mediated hydrogen bond (2)	DC-11	15.19,91.61	-8.94	-13.45 12.17	-2.94 -28.47	-36.6 -25.24
40	72	π- π (2)	DG-10, DC-9	15.12,93.65	-9.45	10.66 12.6	-24.86 -29.45	47.95 -4.95
41	866	π- π (1), Water mediated hydrogen bond (1)	DC-11, DG-10	14.98,92.48	-10.38	-10.31 10.77	5.09 -39.12	-103.44 -40.32
42	205	π- π (1), π-cation (1), Water mediated hydrogen bond (2)	DC-13, DA-17	14.20,87.23	-9.61	4.62 11.78	48.23 -30.66	18.28 -59.66
43	173	Unbound	-	13.77,85.48	-9.61	0.45	-89.97	-179.3

						6.27	-35.65	-124.87
44	206	π- π (1)	DC-13	13.77,88.10	-9.61	4 13.32	89.61 -24.29	82.95 -3.56

Table S3. Type of interactions present in the free energy minimum of L2 ligand

S.No	Frame number	Type of interactions	Interacting residues	Collective variables (Distance (Å) and Angle (°))	Energy Kcal/mol	Rise DT-8 DA17	Roll DT-8 DA17	Twist DT-8 DA17
1	678	Hydrogen bond (1), Water mediated hydrogen bond (1)	DG-10, DG-12	14.14, 99.49	-8.93	8.01 4.41	64.91 -31.47	17.11 -143.6
2	775	Unbound	-	13.95, 95.98	-8.93	-8.17 5.47	-101.98 -20.94	65.06 -141.18
3	768	π- π (1), Hydrogen bond (1)	DT-7	13.32, 97.15	-8.93	0.35 6.33	58.17 -34.27	-173.51 -149.51
4	707	π- cation (1)	DC-21	13.36, 94.53	-8.93	-3.29 1.88	-88.92 -50.83	-129.35 -120.44
5	774	π- π (2), Water mediated hydrogen bond (1)	DC-21	13.17, 93.36	-8.76	-10.66 7.74	-70.09 -71.89	19.71 -151.8
6	704	π- cation (1), Water mediated hydrogen bond (2)	DA-18, DT-19	12.89, 96.28	-8.93	5.94 5.39	121.78 -32.44	39.48 -176.79
7	702	π- cation (1), Hydrogen bond (1)	DA-18, DT-19	12.89, 96.28	-8.76	8.33 5.24	108.28 32.57	22.04 170.19
8	701	π- cation (1)	DA-18	13.44, 90.15	-8.76	-0.56 4.35	92.25 53.26	-160.6 168.26
9	706	Unbound	-	13.71, .86	-8.76	-9.17 -2.49	126.06 8.54	-37.4 -107.73
10	779	π- cation (1), Hydrogen bond (1)	DC-21, DT-7	13.98, 86.06	-8.76	-8.56 7.89	90.83 -50.19	30.02 -139.99
11	69	Hydrogen bond (2)	DG-4	13.95, 85.27	-8.43	6.77 5.31	-49.35 -49.95	83.62 -156.98
12	762	Hydrogen bond (1)	DT-7	13.05, 86.06	-8.76	-2.02 6.3	100.32 -42.33	163.66 -138.62
13	758	π- π (1)	DT-7	12.54, 86.35	-8.76	-6.84 5.82	120.7 -38.05	-70.86 -139.08

14	64	Hydrogen bond (2)	DG-4	12.47, 84.31	-8.43	4.15 5.15	-31.87 -59.95	72.72 -122.1
15	51	Hydrogen bond (2)	DG-4	12.08, 83.73	-8.70	4.12 4.67	-44.9 -62.05	127.6 -117.56
16	729	Unbound	-	12.66, 81.98	-8.43	-4.18 0.02	-120.41 57.09	-75.22 98.6
17	757	π- π (1), π- cation (2), hydrogen bond (1), Water mediated hydrogen bond (1)	DG-4, DT-7, DC-21	11.80, 80.52	-8.70	-4.25 7.03	-109.46 -63.67	139.87 -163.61
18	730	π- π (1)	DT-20	11.65, 84.02	-8.70	1.44 1.56	118.2 45.26	-148.3 110.51
19	756	Unbound	-	10.91, 79.93	-8.70	-6.76 4.97	-99.34 93.8	87.22 169.07
20	765	Hydrogen bond (1)	DT-7	11.34, 77.01	-8.70	-0.36 6.3	107.31 -36.5	-177.97 -148.84
21	49	Hydrogen bond (2)	DG-4	10.56, 75.56	-7.32	6.11 6.02	-68 -74.83	96.48 -157.43
22	81	π- π (1), Hydrogen bond (2)	DG-4, DT-19	10.60, 74.39	-7.32	3.52 5.2	14.88 -77.58	18.3 -141.96
23	50	Hydrogen bond (1)	DG-4	10.17, 71.47	-8.76	5.29 4.74	-35.14 -56.99	83.71 -142.82
24	792	π- π (1)	DT-19	9.97, 68.55	-8.76	-4.17 4.65	-120.37 -43.82	152.19 156.16
25	48	Hydrogen bond (1), Water mediated hydrogen bond (3)	DG-4, DC-3, DA-18	9.70, 70.30	-8.76	6.75 5.35	-64.03 -64.89	90.05 -146.41
26	37	π- π (1), π- cation (1), Hydrogen bond (2), Water mediated hydrogen bond (1)	DA-5, GD-4, Dt-19	9.58, 72.34	-8.76	6.87 7.25	-29.06 -80.87	83.18 -163.16
27	36	π- π (1), π- cation (1), Hydrogen bond (2), Water	DA-5, DG-4, DT-19	9.70, 66.22	-9.11	8.33	-49.18	74.45

		mediated hydrogen bond (1)				5.97	-58.42	-171.85
28	712	π- π (1)	DT-8	9.51, 63.59	-9.11	-8.13 -13.04	-105.92 60.35	54.06 -5.57
29	791	Unbound	-	9.93, 60.96	-9.11	-5.91 4.97	-124.08 -48.01	-11.43 -159.22
30	46	Hydrogen bonding (1), Water mediated hydrogen bond (1)	DG-4	9.66, 57.46	-8.08	5.7 6.16	-50.10 -52.69	92.3 -174.19
31	807	Hydrogen bond (1)	DT-8	8.96, 63.88	-9.11	6.92 4.43	101.14 -36.99	19.53 -148.15
32	800	Hydrogen bond (1), Water mediated hydrogen bond (1)	DT-8, DT-7	8.86, 66.51	-9.11	-6.44 5.24	-116.36 -59.89	-71.32 -150.95
33	713	π- π (1), Water mediated hydrogen bond (1)	DT-8	8.49,5.34	-9.11	-4.03 -8.71	-100.06 40.4	136.47 -50.26
34	797	Hydrogen bond (1)	DT-8	8.42, 63.01	-9.11	2.75 4.75	106.22 -51.28	149.06 -168.34
35	710	π- π (1)	DT-8	8.18, 65.34	-9.11	8.27 -0.19	79.95 -10.69	-45.02 -123.43
36	45	π- cation (1), Hydrogen bond (1), Water mediated hydrogen bond (1)	DA-17, DG-4	8.77, 63.01	-9.11	2.45 6.29	9.6 -68.84	-8.35 -164.87
37	713	π- π (1), Water mediated hydrogen bond (2)	DT-8	8.42, 65.05	-9.11	-4.03 -8.71	-100.06 40.4	136.47 -50.26
38	137	π- π (1), Water mediated hydrogen bond (1)	DT-7	8.26, 58.34	-8.08	-8.87 5.76	-40.37 -50.12	-64.2 -159.28
39	799	Hydrogen bond (2)	DT-8	7.75, 57.46	-7.47	-8.06 4.57	-110.46 -54.26	15.92 -139.12

40	806	Hydrogen bond (1), Water mediated hydrogen bond (1)	DT-8	8.42, 54.83	-8.08	-5.43 5.1	-115.2 -60.77	-39.93 -173.17
41	802	π- π (1), Hydrogen bond (3)	DT-8	7.09, 57.75	-7.47	-6.52 4.89	-109.51 -55.17	90.04 -167.97
42	39	π- cation (1), Hydrogen bond (3)	DG-4, DA-5, DT-7	7.56, 62.13	-8.77	6.39 7.28	-16.65 -44.06	64.81 -174.25
43	798	Hydrogen bond (1)	DT-8	7.32, 61.84	-8.77	4.85 5.48	107.9 -52.66	68.24 117.57
44	41	π- cation (1), Hydrogen bond (2)	DA-17, DG-4	6.90, 58.92	-8.77	7.06 7.5	-16.99 55.61	51.43 172.73
45	803	π- π (1), Hydrogen bond (1), Water mediated hydrogen bond (1)	DT-8, DT-7	6.74, 61.84	-8.77	7.63 5.35	116.22 -50.97	20.99 -174.86
46	34	π- π (2), π- cation (1), Hydrogen bond (3)	DG-4, DT-19, DA-17	7.29, 68.55	-7.18	8.05 10.37	-87.48 22.38	11.4 69.33
47	804	π- π (1), Hydrogen bond (2), Water mediated hydrogen bond (1)	DG-4, DT-8, DT-7	7.56, 73.32	-7.18	-6.06 4.57	-130.97 -53.78	51.8 -155.29
48	750	Water mediated hydrogen bond (1)	DT-7	8.53, 78.77	-10.15	-8.33 4.9	-98.9 -33.69	-21.98 -135.09
49	32	π- π (2), π- cation (1), Hydrogen bond (3)	DG-4, DT-19, DT-20	8.26, 87.52	-9.46	2.7 8.85	-2.37 33.17	12.91 133.54
50	20	π- π (1), Water mediated hydrogen bond (1)	DT-20, DT-19	8.22, 81.98	-10.45	2.86 5.97	5.93 -34.85	-3.71 -178.47
51	805	π- cation (1), Hydrogen bond (2)	DC-21, DT-8	8.22, 83.43	-10.45	-3.42 4.91	-125.2 42.5	141.95 178.42
52	718	π- cation (1)	DC-21	9.58, 72.77	-9.46	-6.93 -8.49	-91.42 15.1	78.99 -51.72
53	743	π- π (1)	DT-7	10.60, 93.94	-9.43	-3.84	-88.8	143.05

						8.14	45.94	131.97
54	766	π- π (1), Water mediated hydrogen bond (1)	DT-7, DT-8	11.49, 93.36	-9.43	-4.54 6.28	-106.83 -42.17	126.72 -129.32
55	724	Unbound	-	11.88, 91.90	-9.43	-8.58 -2.28	-109.37 68.42	4.82 100.2
56	708	Unbound	-	11.88, 89.86	-9.43	-1.91 3.81	-112.25 -75.16	-112.34 -124.23
57	709	Unbound	-	11.49, 96.57	-7.54	5.87 0.85	117.48 -35.8	71.43 -105.43
58	778	π- cation (1)	DC-21	12.00, 96.28	-7.54	3.64 4.97	103.95 -39.71	-120.34 -140.8
59	732	π- cation (1)	DC-21	11.84, 96.86	-7.54	-6.38 3.83	-103.05 59.37	86.18 108.56
60	38	π- π (2), Hydrogen bond (3), Water mediated hydrogen bond (1)	DG-4, DT-19, DT-20	9.31, 89.10	9.73	7.74 5.26	-37.71 -73.93	74.16 -135.15
61	759	Hydrogen bond (2)	DT-8	10.48, 87.52	-9.43	-8.7 5.88	-110.23 -57.14	26.57 -124.53
62	723	Unbound	-	10.52, 82.27	-8.70	8.58 1.44	85.31 87.76	37.12 132.47
63	754	Unbound	-	9.97, 83.73	-10.45	-9.24 6.83	-100.73 -78.42	37.3 -156.86
64	715	Unbound	-	9.51, 90.73	-9.73	7.23 -10.01	111.91 24.13	74.2 -14.31
65	711	π- π (1)	DT-8	9.43, 79.64	-10.45	-4.92 -14.16	-111.3 46.76	99.11 -0.12
66	754	Unbound	-	9.39, 80.52	-10.45	5.84 6.83	-135.31 -78.42	-64.2 -156.86

Table S4. Type of interactions present in the free energy minimum of L3 ligand

S.No	Frame number	Type of interactions	Interacting residues	Collective variables (Distance (Å) and Angle (⁰))	Energy kcal/mol	Rise	Roll	Twist
1	982	Water mediated hydrogen bond (1)	DT-20	21.08, 98.61	-7.59	3.11 -7.35	33.99 77.03	157.71 -20.84
2	893	π- π (1)	DT-19	22.15, 96.28	-7.59	7.4 -7.84	57.01 76.78	-12.81 -27.34
3	910	Hydrogen bond (2)	DT-20, DG-24	21.14, 94.82	-9.77	7.04 -9.27	48.14 75.48	2.51 28
4	985	π- π (3)	DT-19, DT-20	21.53, 91.31	-10.09	7.6 -7.97	54.01 60.82	74.83 -18.26
5	913	π- π (1)	DT-20	20.59, 82.85	-9.07	2.42 -7.76	97.76 73.28	-58.3 -14.87
6	949	π- π (2), Hydrogen bond (1)	DT-19	20.70, 81.68	-9.07	4.97 -9.56	52.03 91.15	98.88 -26.78
7	497	Unbound	-	21.72, 79.35	-7.92	4.37 -0.12	8.18 -152.09	2.23 135.77
8	474	Unbound	-	20.90, 77.01	-7.56	7.38 -7.71	80.32 96.25	60.39 1.07
9	796	π- π (1), π-cation (1)	DC-9, DT-19	21.21, 75.56	-7.74	-2.75 -10.16	30.4 112.98	-18.03 -68.19
10	505	Unbound	-	21.33, 74.68	-7.74	2.92 -12	-22.52 133.93	4.1 -29.64
11	234	π- π (1)	DT-19	20.32, 74.39	-7.56	4.23 -12.32	63.7 150.84	51.05 -64.34
12	277	Unbound	-	20.59, 73.22	-7.68	-1.51 -8.21	35.18 63.69	-22.69 7.12
13	74	Unbound	-	19.61, 77.01	-7.69	2.05 -10.18	91.63 110.56	119.44 -12.01
14	473	Unbound	-	19.61, 77.01	-7.69	6.51 -8.28	80.94 68.32	74.6 -2.63

15	62	Unbound	-	19.22, 74.10	-8.21	6.09 -9.95	49.94 102.57	39.74 -96.42
16	242	Unbound	-	19.92, 70.01	-8.72	8.98 -10.31	28.97 147.39	17.25 -0.76
17	829	π- π (1)	DT-20	19.69, 69.13	-8.72	-6.85 2.78	19.85 157.19	-17.84 168.69
18	964	π- π (2)	DT-19	19.69, 67.97	-8.72	6.39 -10.12	90.79 97.95	-52.88 -21.06
19	235	Unbound	-	20.00, 67.68	-8.72	6.48 -8.32	38.08 106.33	-19.4 15.7
20	840	π- π (2), Hydrogen bond (2), Water mediated hydrogen bond (1)	DT-20, DG-24	-19.33, 68.55	-8.72	-9.51 -4.13	5.06 138.89	37.82 93.31
21	881	Unbound	-	20.70, 66.80	-8.01	-5.44 -3.92	-21.22 43.34	-9.48 43.99
22	901	π- π (1), Hydrogen bond (2)	DT-19, DT-20, DG-24	21.18, 65.63	-7.64	-0.69 -8.9	93.85 82.93	37.45 -58.15
23	895	Unbound	-	18.55, 77.01	-8.21	7.31 -8.62	65.46 108.14	0.75 -13.74
24	240	Unbound	-	19.06, 61.25	-7.94	6.48 -8.32	38.08 106.33	-19.4 15.7
25	958	π- π (1), Hydrogen bond (1), Water mediated hydrogen bond (1)	DT-19, DT-7	19.14, 58.34	-7.83	7.81 -7.79	37.35 73.03	81.15 -17
26	963	π- π (2)	DT-20, DT-19	18.43, 62.13	-7.94	8.18 -11.04	53.9 103.87	82.69 -29.79
27	241	Unbound	-	17.92, 67.38	-8.41	7.48 -8.07	18.07 122..14	-25.03 24.33
28	479	Water mediated hydrogen bond (1)	DG-14	17.84, 63.01	-8.75	-0.32 -10.26	86.96 111.93	-2.5 -64.55

29	815	π- π (1)	DT-20	17.77, 62.71	-8.67	-2.48 -5.75	14.45 47.23	6.73 11.12
30	496	π- π (1)	DC-13	17.34, 62.13	-8.19	2.98 -7.04	-34.43 132.49	9.02 -154.58
31	63	Water mediated hydrogen bond (1)	Dt-20	17.96, 58.63	-7.99	6.3 -13.29	50.5 106.93	49.5 -60.04
32	61	π- π (1)	DC-3	17.73, 56.00	-7.99	3.4 -5.15	6.34 36.32	49.92 -133.28
33	827	π- π (1)	DT-20	17.53, 57.75	-7.99	-3.95 3.29	-12.63 -147.77	-7.77 -174.36
34	231	Unbound	-	17.37, 78.77	-7.59	5.82 -7.89	-28.08 84.88	-16.32 -125.62
35	480	Water mediated hydrogen bond (1)	DC-13	17.30, 70.01	-8.50	-3.81 -8.89	31.65 115.68	6.62 -17.63
36	710	π- π (1), Hydrogen bond (1)	DT-8, DT-20	16.94,65.63	-8.42	9.85 -10.39	51.91 113.69	11.49 -94.68
37	721	Unbound	-	16.24, 66.22	-8.88	8.79 -7.59	52.56 85.38	9.43 -45.17
38	724	π- π (1)	DT-19	15.89, 60.96	-8.42	0.13 -9.04	57.67 89.31	-179.25 0.31
39	229	π- π (1)	DT-19	15.77, 63.59	-8.88	7.51 -11.45	60.78 122.78	67.29 -58.31
40	478	Unbound	-	15.53, 62.42	-7.60	-2.03 -8.61	26.37 89.25	10.71 -26.24
41	969	π- π (1)	DT-20	15.49, 66.22	-8.17	2.72 -9.41	48.06 70.64	167.04 3.33
42	836	π- π (1), Hydrogen bond (1)	DT-19 DG-24	15.46, 68.84	-8.36	-8.47 -2.17	-54.25 146.58	55.33 116.54
43	720	Unbound	-	14.91, 67.09	-8.36	8.52 -11.09	57.09 113.92	35.06 -44.31
44	714	π- π (1)	DT-20	16.47, 81.39	-7.77	9.48 -10.4	24.27 120.04	8.86 -46

45	221	Unbound	-	16.43, 80.52	-8.18	9.09 -11.17	55.61 122.22	14.52 -98.33
46	970	π- π (1), Water mediated hydrogen bond (1)	DT-20	16.67, 76.14	-7.86	8.2 -7.79	46.26 56.84	35.81 11.53
47	826	π- π (1), Hydrogen bond (1)	DT-19, DG-24	16.47, 75.85	-8.65	0.34 10.68	85.12 -127.26	-83.34 -78.7
48	832	Unbound	-	16.16, 76.43	-8.65	-3.77 -3.83	-14.34 126.52	-4.03 69.53
49	831	π- π (1)	DT-20	15.69, 79.53	-8.18	-2 -2.85	3.75 147.16	-1.06 93.43
50	476	Unbound	-	15.57, 81.39	-7.44	2.98 -7.04	-34.43 132.49	9.02 -154.58
51	738	π- π (1)	DT-20	15.38, 81.10	-7.44	8.3 -6.49	67.72 78.7	27.26 -25.31
52	66	Hydrogen bond (1)	DT-19	14.40, 80.81	-7.64	259 8.91	-107.03 -174.92	-176.24 -124.16
53	477	Unbound	-	14.51, 77.89	-7.64	-1.71 -7.16	-2.43 58.44	-22.81 -15.2
54	716	π- π (1)	DG-24	14.01, 70.59	-8.27	9.62 -9	47.83 99.03	16.55 -15.93
55	824	Unbound	-	14.48, 73.22	-8.27	-7.02 7.66	-37.92 -169.89	13.6 -129.95
56	502	Unbound	-	14.48, 70.89	-8.27	3.65 -8.27	-3.61 140.81	1.07 147.86
57	973	π- π (1)	DG-24	14.01, 70.59	-8.24	5.31 -8.4	43.77 72.22	134.02 1.11
58	725	Unbound	-	13.85, 67.68	-8.05	8.64 -7.96	52.19 63.31	30.11 -29.44
59	830	Unbound	-	14.12, 75.26	-7.97	-3.63 -6.33	-2.95 153.48	-16.28 60.96
60	718	Unbound	-	13.85, 74.68	-7.98	8.66 -9.92	61.56 111.44	-1.32 -24.83

61	978	Unbound	-	73.57, 75.85	-7.92	8.32 -10.94	57.75 105.99	44.37 -62.88
62	65	Unbound	-	13.26, 72.05	-8.26	1.76 -10.65	118.41 103.55	48.7 -68.84
63	732	Unbound	-	13.18, 79.93	-7.91	8.92 -5.44	23.77 63.66	0.26 -3.78
64	744	π- π (2)	DT-19	13.07, 81.98	7.86	1.5 -10.28	-66.32 98.36	-179.06 -27.95
65	785	Unbound	-	12.60, 81.10	-8.07	-3.89 -10.09	-15.32 98.9	-3.72 -36.12
66	835	π- π (1), Hydrogen bond (1), Water mediated hydrogen bond (1)	DT-20, DG-24	12.83, 83.73	-8.07	-9.74 -2.91	-4.95 156.96	37.06 125.6
67	745	Unbound	-	13.89, 88.69	-8.00	7.64 -9.84	63 91.84	49.44 27.08
68	199	Unbound	-	13.26, 88.40	-8.00	2.28 -12.23	37 122.06	-27.06 -34
69	499	unbound	-	12.91, 90.15	7.82	2.35 8.42	-21.47 -155.79	8.28 29.25
70	743	π- π (2)	DT-19	13.14, 92.77	-8.25	9.23 -10.06	54.22 94.15	21.29 -47.37
71	198	Water mediated hydrogen bond (1)	DG-24	13.10, 94.23	-8.25	7.49 -11.94	20.01 131.5	7.11 -92.17
72	745	Unbound	-	13.97, 88.98	-7.80	7.64 -9.84	63 91.84	49.44 -27.08
73	711	Unbound	-	13.73, 91.90	-8.25	8.73 -10.43	39.24 104.4	9.8 -82.51
74	729	π- π (1)	DT-19	13.93, 94.23	-7.88	8.45 -4.55	66.36 70.41	23.57 17.3
75	500	Unbound	-	13.34, 99.78	-8.07	3.97 -8.95	-5.23 144.51	13.38 -141.29

76	713	π- π (1)	DT-20	14.91, 89.86	-7.57	8.18 -9.72	61.57 113.01	30.92 -66.02
77	712	Unbound	-	15.06, 91.69	-7.58	8.71 -9.76	49.13 114.52	44.73 -78.25
78	222	π- π (2)	DT-19, DT-20	14.87, 93.36	-7.58	7.92 -11.67	47.12 113.09	26.11 -50.8
79	213	π- π (2), Water mediated hydrogen bond (1)	DT-19	14.79, 95.40	-7.86	6.94 -10.99	41.41 138.55	19.79 -97.37
80	68	π- π (1), Water mediated hydrogen bond (1)	DC-21, DT-19	14.48, 96.28	-7.86	3.36 -9.43	-97.03 126.94	-158.97 -2.5
81	73	Water mediated hydrogen bond (1)	DG-2	20.12, 80.52	-6.95	3.12 -10.66	-100.49 107.51	-168.56 -44.28

Table S5. Rise, roll and twist of DC-11 residue in intercalation and unbound state of L1 ligand

Frame number of intercalated snapshot	**Rise**	**Roll**	**Twist**
71	5.49	-38.51	57.87
72	10.66	-24.86	47.95
205	4.62	48.23	18.28
206	4	89.61	82.95
208	2.51	94.13	57.17
849	-12.5	-6.93	-52.26
860	-10.08	-6.41	-89.8
861	-7.52	-38.32	-89.86
850	-13.45	-2.94	-36.6
Frame number for unbounded snapshot	**Rise**	**Roll**	**Twist**
73	8.81	-11.75	31.39
172	2.23	89.08	122.4
169	1.62	66.52	75.88
171	3.74	81.04	128.94
173	0.45	-89.97	-179.3
442	-7.6	-3.05	-108.5
889	-7.5	-28.63	-98.86
864	-9.03	-12.61	-112.68
859	-8.55	-18.71	-86.58

Table S6. Rise, roll and twist of DC-11 residue in intercalation and unbound state of L2 ligand

Frame number of intercalated snapshot	**Rise**	**Roll**	**Twist**
36	8.33	-49.18	74.45
37	6.87	-29.06	83.18
678	8.01	64.91	17.11
701	-0.56	92.25	-106.6
702	8.33	108.28	22.04
704	5.94	121.78	39.48
712	-8.13	-105.92	54.06
713	-4.03	-100.86	136.47
757	-4.25	-109.46	139.87
779	-8.56	90.83	30.02
Frame number for unbounded snapshot	**Rise**	**Roll**	**Twist**
706	-9.17	26.06	-37.4
708	-1.91	-112.85	-112.34
709	5.87	117.48	71.43
715	7.23	111.91	74.2
723	8.58	85.31	37.12
724	-8.58	-107.37	4.82
729	-4.18	-120.41	-75.22
756	-0.36	107.31	177.97
775	-8.17	-101.98	65.06
791	-5.91	-124.08	-11.43

Table S7. Rise, roll and twist of DC-11 residue in intercalation and unbound state of L3 ligand

Frame number of intercalated snapshot	**Rise**	**Roll**	**Twist**
234	4.23	63.7	51.03
710	9.85	51.91	11.49
724	8.79	52.56	9.43
796	-2.75	30.4	-18.03
829	-6.85	19.85	-17.84
893	7.4	57.01	74.83
913	2.42	97.76	-58.3
949	4.97	52.03	98.88
958	7.81	37.35	81.15
964	6.39	90.79	-52.88
985	7.6	54.01	74.83
Frame number for unbounded snapshot	**Rise**	**Roll**	**Twist**
74	2.05	91.63	119.44
240	6.48	38.08	-19.4
241	7.48	18.07	-25.03
277	-1.51	35.18	-22.69
473	6.51	80.94	74.6
474	7.38	80.32	60.39
497	4.37	8.18	2.23
505	2.92	-22.52	4.1
881	-5.44	-21.22	-9.48
895	7.31	65.96	0.75