Electronic Supplementary Material (ESI) for MedChemComm.

Cellular imaging and mitochondria targeted photo-cytotoxicity in visible light by singlet oxygen using a BODIPY-appended oxovanadium(IV) DNA crosslinking agent

Arun Kumar,[a] Akanksha Dixit,[b] Samya Banerjee,[a] Arnab Bhattacharyya,[a] Aditya Garai,[a] Anjali A. Karande,*[b] and Akhil R. Chakravarty*[a]

[a] Department of Inorganic and Physical Chemistry, Indian Institute of Science and [b] Department of Biochemistry, Indian Institute of Science, Bangalore 560012, India. 560012, India. E-mail: arc@ipc.iisc.ernet.in; anjali@biochem.iisc.ernet.in

Electronic Supplementary Information (ESI)

Experimental Section

Materials and measurements

All the reagents and chemicals were procured from commercial sources (s.d. Fine Chemicals, India; Aldrich, USA) and used as such. Solvents were purified by standard procedures.[S1] Dulbecco's Modified Eagle's medium (DMEM), ethidium bromide, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), annexin V-FITC and agarose (molecular biology grade) were procured from Sigma-Aldrich (USA). Supercoiled (SC) pUC19 DNA (cesium chloride purified) was from Bangalore Genie (India). The ligands, *viz.*, BODIPY and benzyldipicolylamine were prepared by reported procedures.[S2] Synthesis of the complexes was carried out under nitrogen atmosphere using Schlenk technique. Tetrabutylammonium perchlorate (TBAP) was prepared using tetrabutylammonium bromide and perchloric acid.

The elemental analysis was done using a Thermo Finnigan FLASH EA 1112 CHNS analyzer. The infrared and electronic spectra were recorded on Perkin Elmer Lambda 35 and Perkin Elmer spectrum one 55, respectively, at 25 °C. Molar conductivity measurements were done using a Control Dynamics (India) conductivity meter. Electrochemical measurements were made at 25 °C on an EG&G PAR model 253 VersaStat potentiostat/galvanostat with electrochemical analysis software 270 using a three electrode setup consisting of a glassy carbon working, platinum wire auxiliary and a saturated calomel reference electrode (SCE) in DMF. TBAP (0.1 M) was used as a supporting electrolyte for the electrochemical measurements. Electrospray ionization (ESI) mass spectral measurements were made using Bruker Daltonics make Esquire 300 Plus ESI model. Magnetic susceptibility measurements at 298 K were carried out with solid sample using MPMS SQUID VSM (Quantum Design, USA). Flow cytometric analysis was performed using FACS Calibur (Becton Dickinson (BD) cell analyzer) at FL2

channel (595 nm). Confocal microscopy was done using confocal scanning electron microscope (Leica, TCS SP5 DM6000).

Solubility and stability

The complexes displayed good solubility in DMF, DMSO, methanol, ethanol and acetonitrile. They had poor or less solubility in hydrocarbons. The complexes were found to be stable in the monocationic form due to dissociation of the chloride and the solution stability of the cationic species was ascertained from the ESI-MS and the molar conductivity data.

Computational methodology

To rationalize the photophysical properties of the complexes, computational studies were performed for both the complexes using B3LYP/LANL2DZ level of DFT.[S3] The hybrid U3LYP functional and LANL2DZ basis set were used in all calculations as incorporated in Gaussian 09 package.[S4] Visualizations of the optimized structures and the MOs were performed using Gaussview5.0. To ascertain stationary points, further frequency test was performed.

References

(S1) D. D. Perrin, W. L. F. Armarego and D. R. Perrin, *Purification of Laboratory Chemicals*; Pergamon Press: Oxford, 1980.

(S2) (a) P. C. Kunz, N. E. Brückmann and B. Spingler, *Eur. J. Inorg. Chem*., **2007**, 394-399. (b)B. W. Michel, A. R. Lippert and C. J. Chang, *J. Am. Chem.Soc*., **2012**, 134, 15668–15671.

(S3) (a) A. D. Becke, *Phys. Rev*., 1998, **A38**, 3098-3100; (b) A.D. Becke, *J. Chem. Phys*., 1993, **98**, 5648-5652; (c) C. Lee, W. Yang and R.G. Parr, *Phys. Rev., B*. 1988, **37**, 785-789; (d) P.J. Hay and W.R. Wadt, *J. Chem. Phys*., 1985, **82**, 284-298.

(S4) Gaussian 09, Revision A.02, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H.Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L.Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T.Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery, Jr., J. E. Peralta, F.Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, R. Kobayashi,J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi,N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo,R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W.Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J.Dannenberg, S. Dapprich, A. D. Daniels, O. Farkas, J. B. Foresman, J. V. Ortiz, J.Cioslowski, and D. J. Fox, Gaussian, Inc., Wallingford CT, 2009.

Table 1: Selected bond distances (Å) and angles (°) for [VO(benzyldipicolylamine)Cl_2] (**1**) with e.s.d.s in the parentheses

V(1)-O(1)	1.590(2)	V(2)-O(2)	1.588(2)
V(1)-N(1)	2.390(2)	V(2)-N(4)	2.389(2)
V(1)-N(2)	2.137(3)	V(2)-N(5)	2.144(3)
V(1)-N(3)	2.138(3)	V(2)-N(6)	2.136(3)
V(1)-Cl(1)	2.3459(10)	V(2)-Cl(3)	2.3457(11)
V(1)-Cl(2)	2.3517(10)	V(2)-Cl(4)	2.3450(11)
Cl-(1)-V(1)-Cl(2)	93.53(4)	Cl(3)-V(2)-Cl(4)	96.83(4)
O(1)-V(1)-N(1)	164.30(12)	O(2)-V(2)-N(4)	165.01(12)

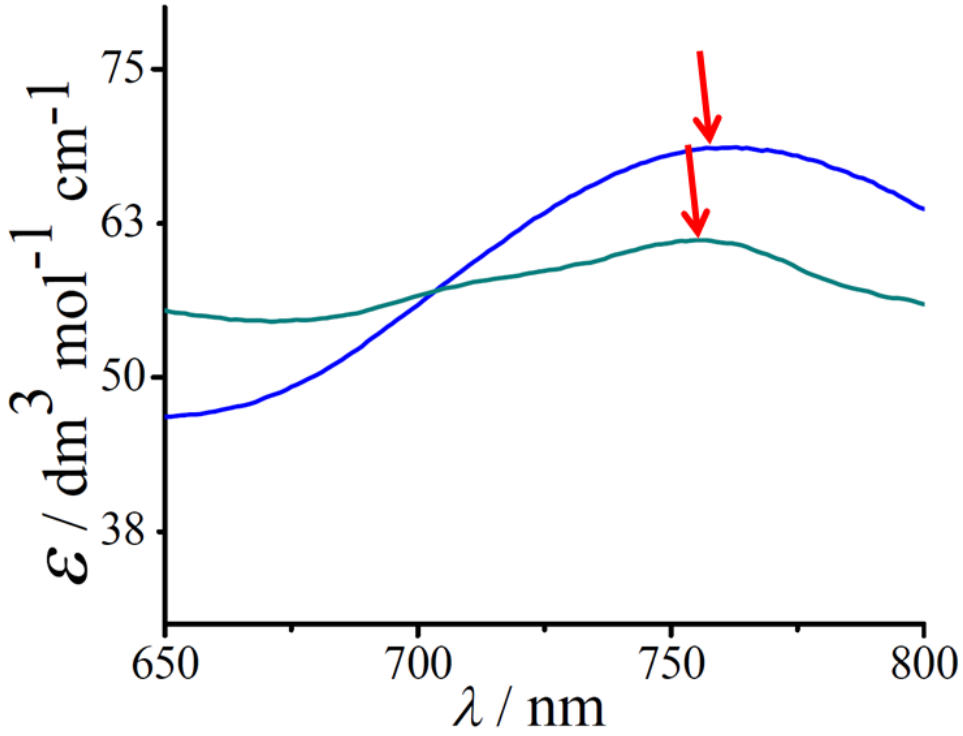

Fig S1. Absorption spectra of the complexes **1** (—) and **2** (—), showing the d-d band, in 20% aqueous DMF.

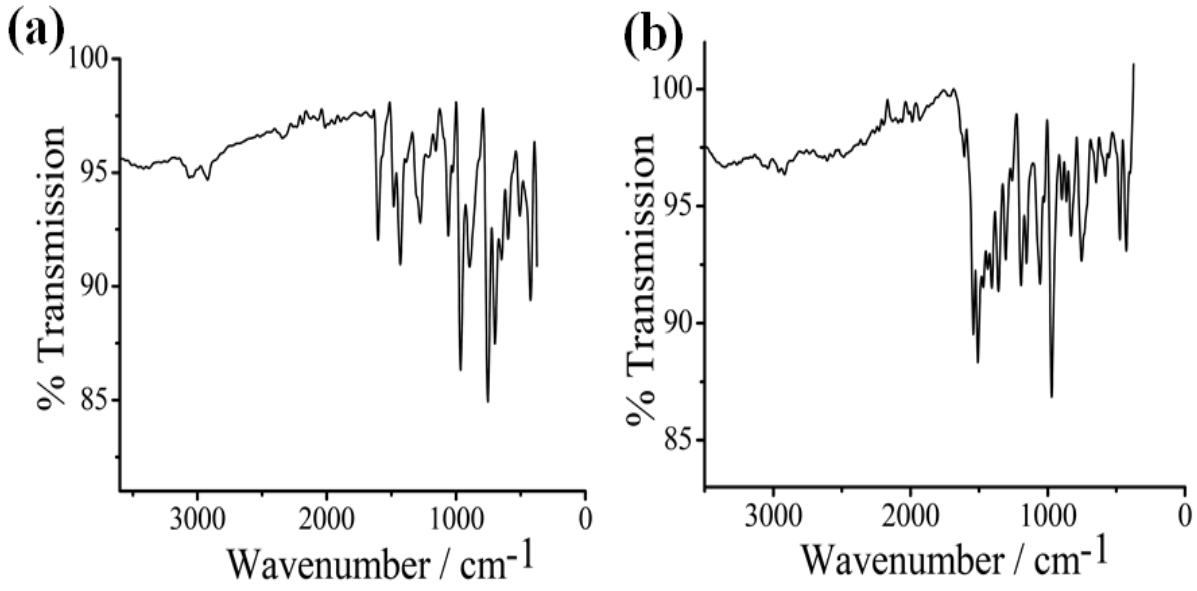

Fig S2. IR spectra of the complexes **1** (a) and **2** (b).

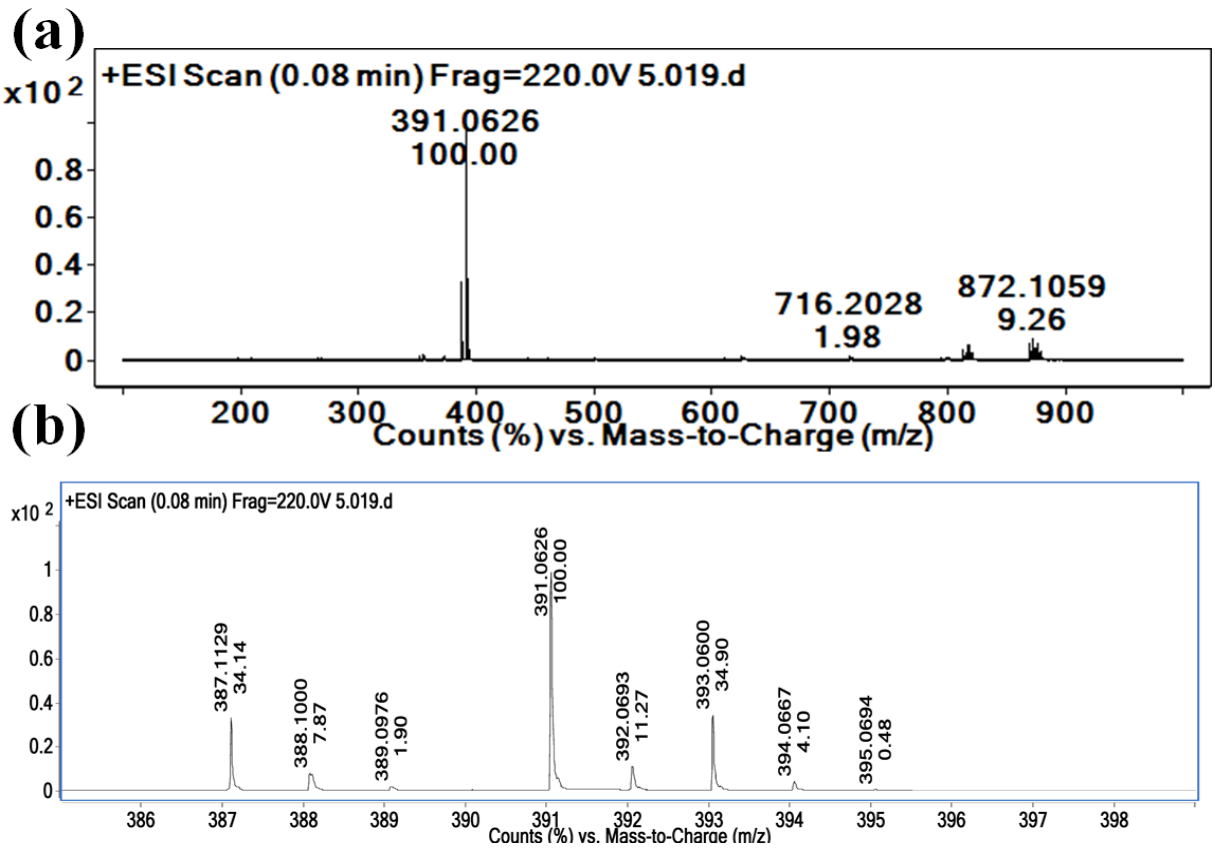

Fig S3. The ESI-MS spectrum of complex **1** showing the prominent $[M\text{-}Cl]^+$ peak in methanol (b) The isotropic distribution of the molecular ion peak.

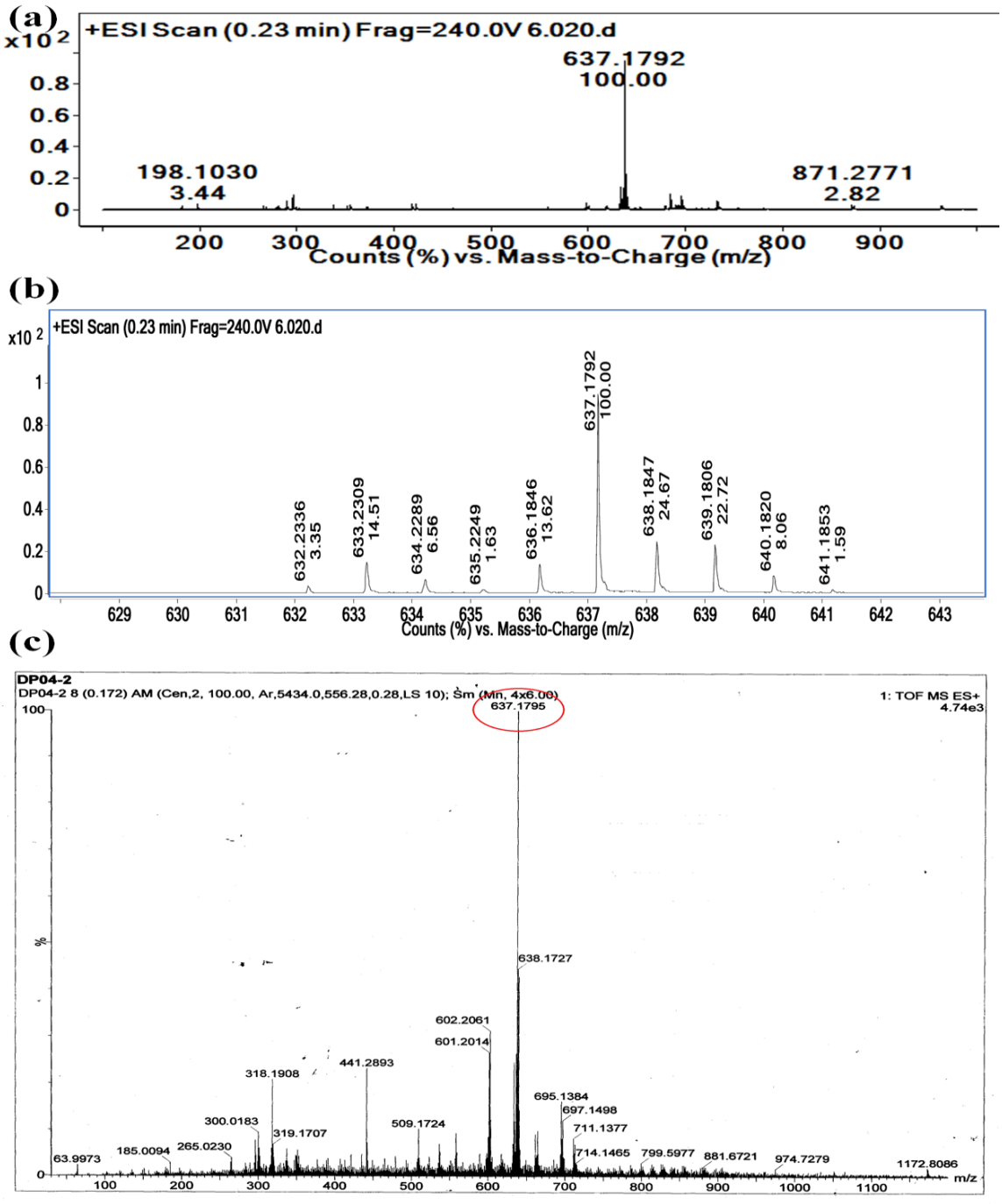

Fig S4. (a) The ESI-MS spectrum of complex **2** showing the prominent $[M\text{-}Cl]^+$ peak in methanol. (b) The isotropic distribution of the molecular ion peak. (c) The HRMS spectrum showing the prominent $[M\text{-}Cl]^+$ peak at 637.1795 (m/z) in methanol.

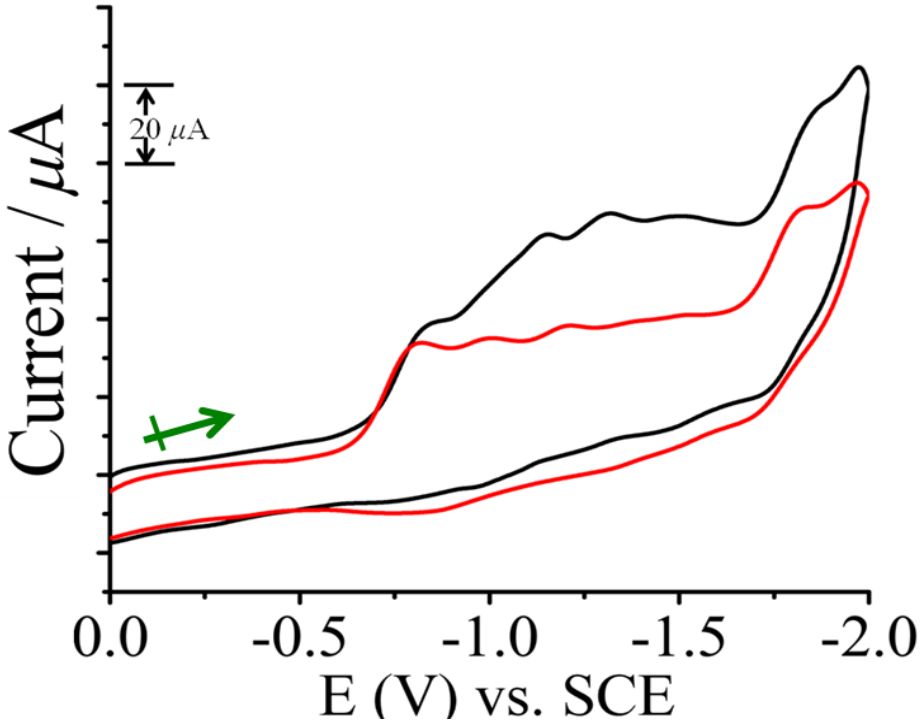

Fig. S5. Cyclic voltammograms of the complexes **1** (—) and **2** (—) showing the cathodic response in DMF at a scan rate of 50 mV s^{-1} and 0.1 mol TBAP as the supporting electrolyte.

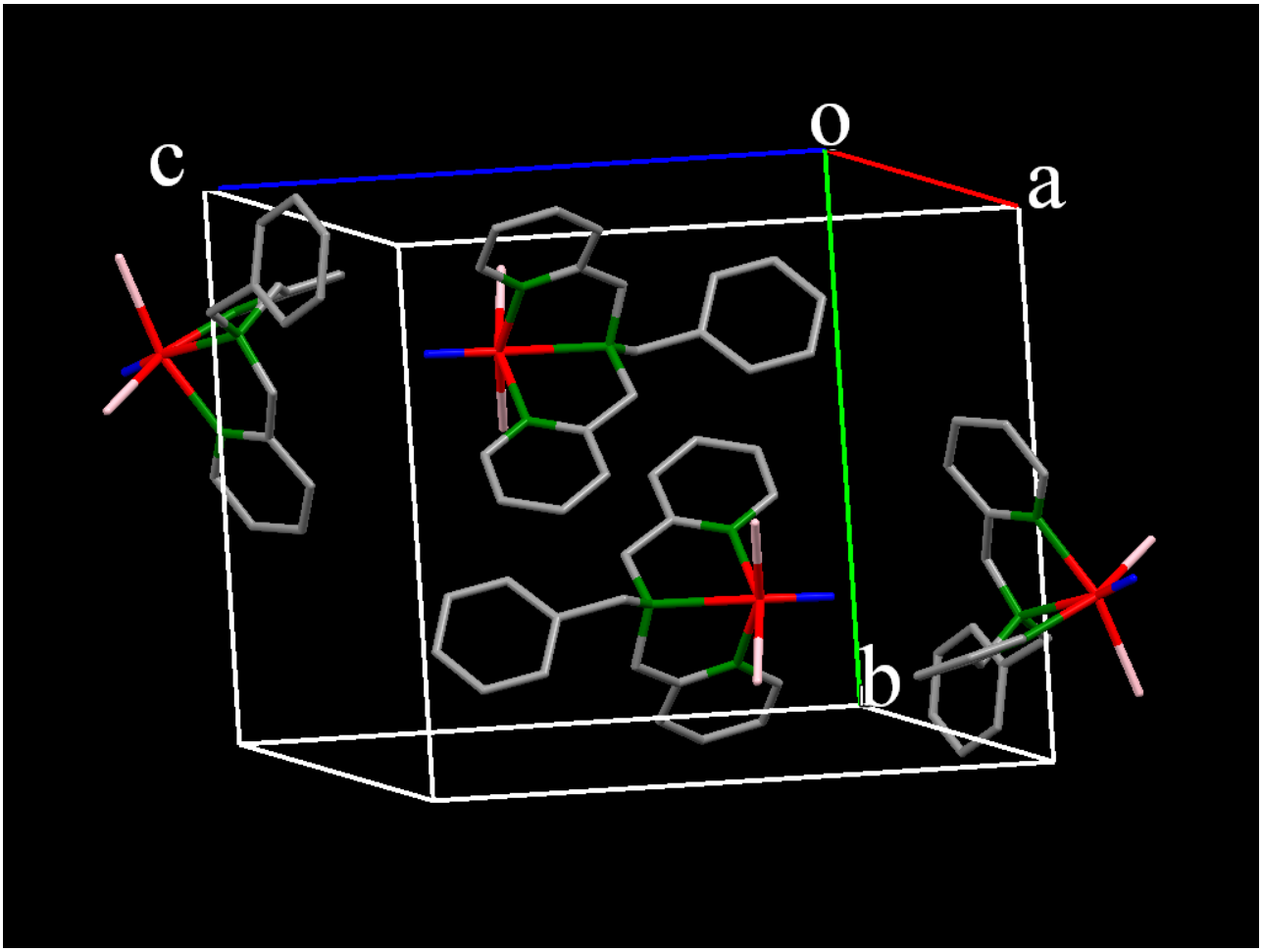

Fig. S6. Unit cell packing diagram of Complex **1**. [color codes: C grey, N green, O blue, V red, Cl pink]

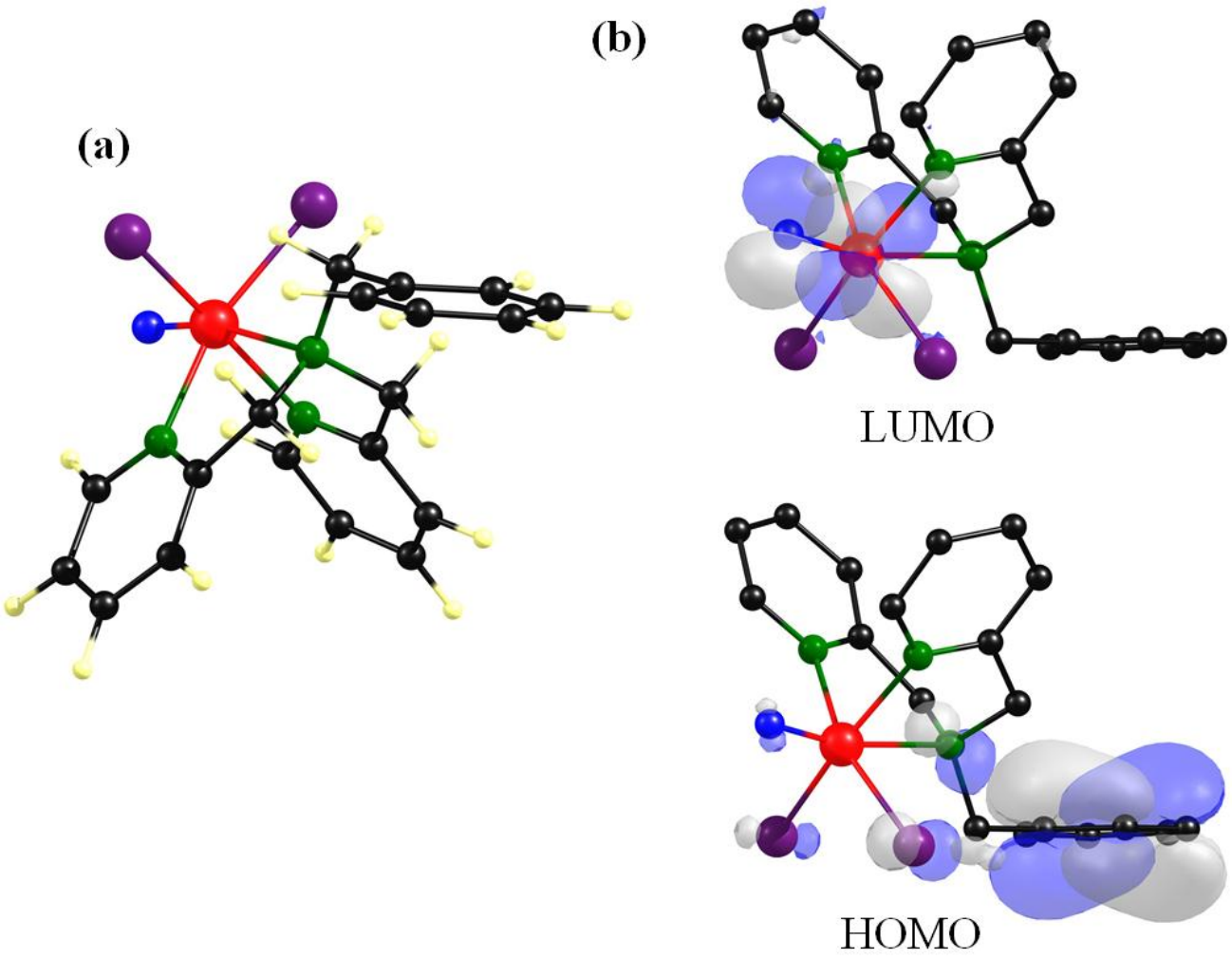

Fig. S7. (a) The energy-optimized structure of the complex **1** [color codes: C black, N green, O blue, V red, Cl purple and H yellow]. (b) The HOMO and LUMO of complex **1**.

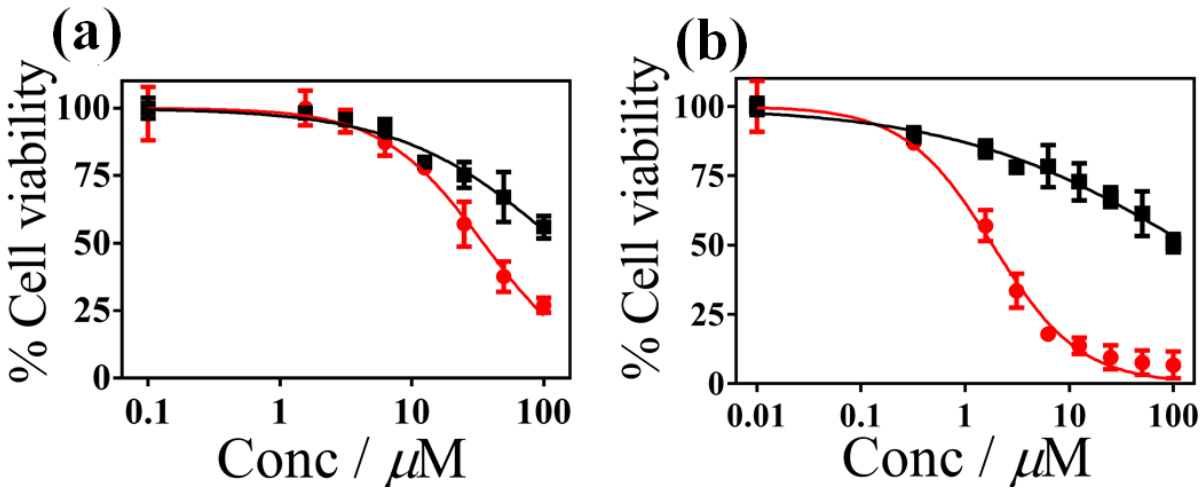

Fig. S8. Photocytotoxicity of the complexes (a) Complex **1** and (b) Complex **2**. in HeLa cell lines on 4 h incubation in dark followed upon photo-irradiation in visible light (400 to 700 nm) for 1 h as determined by MTT assay. The photo-exposed and dark-treated cells are shown in red and black colour symbols, respectively.

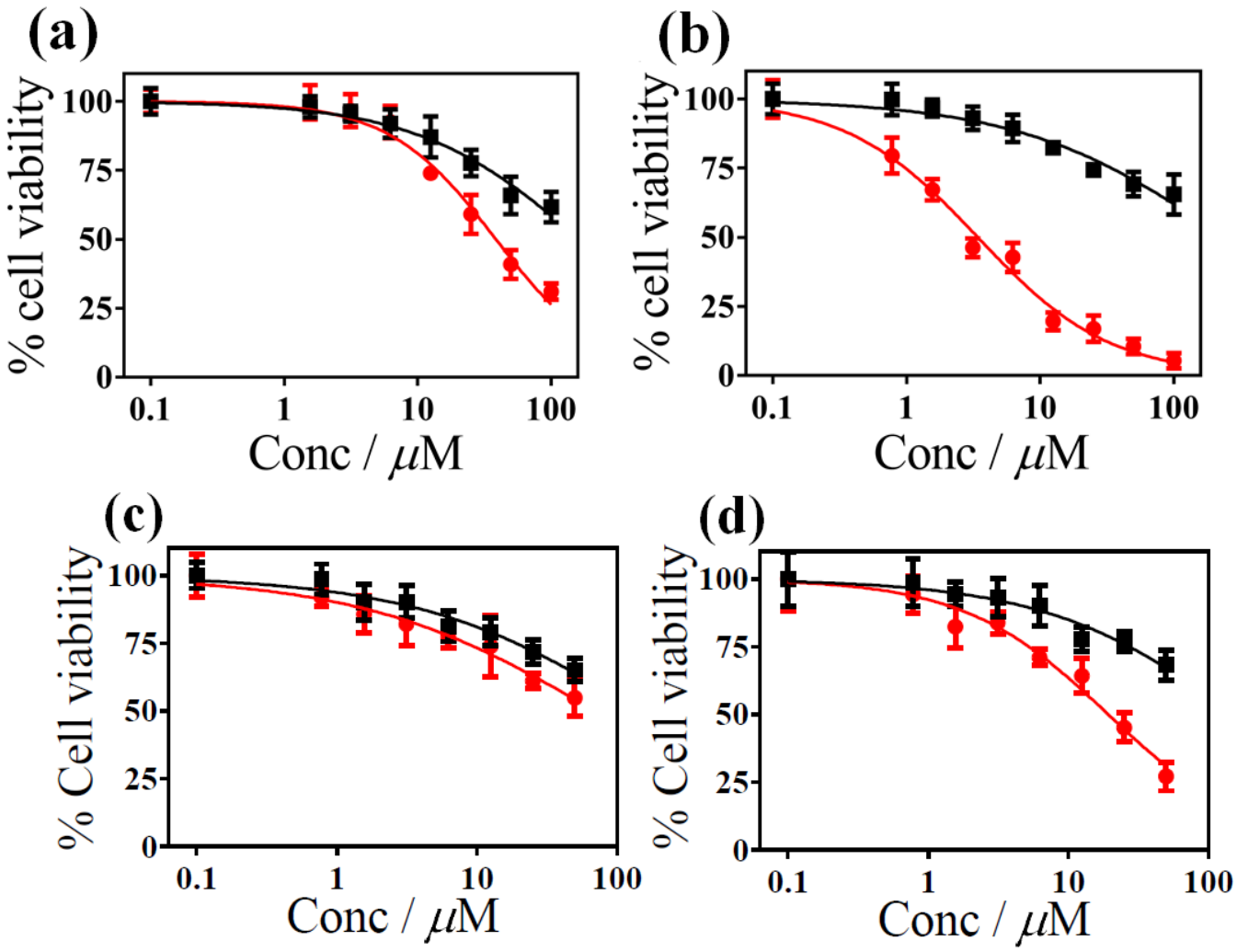

Fig. S9. Photocytotoxicity of the complexes (a) Complex **1** and (b) Complex **2** in MCF-7 cell lines (c) Complex **1** and (d) Complex 2 in MCF-10 A cell lines, on 4 h incubation in dark followed upon photo-irradiation in visible light (400 to 700 nm) for 1 h as determined by MTT assay. The photo-exposed and dark-treated cells are shown in red and black colour symbols, respectively.

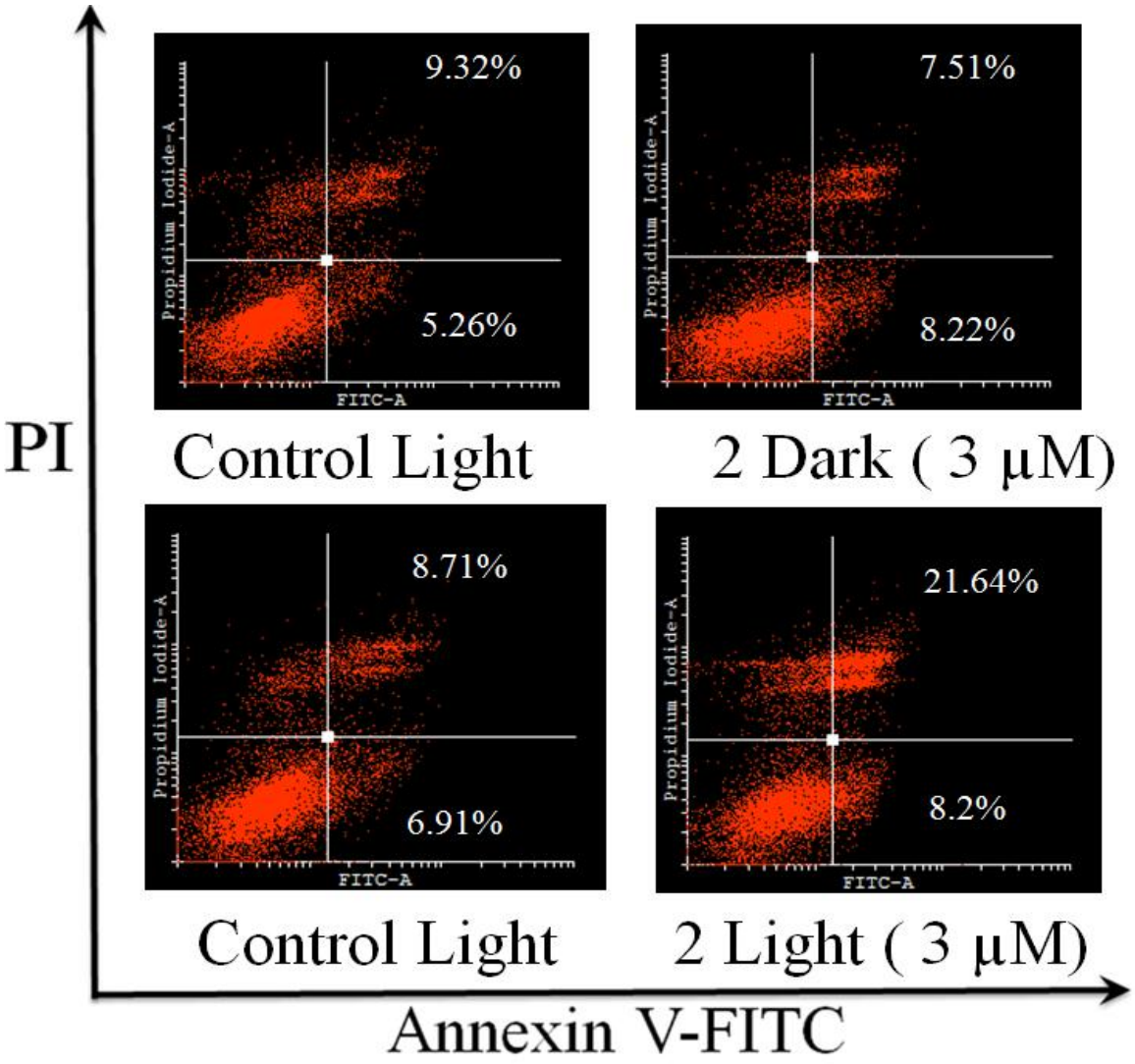

Fig. S10. FACScan profiles of Annexin-V FITC and propidium iodide (PI) staining of HeLa cells undergoing apoptosis induced by complex **2** in dark and in visible light (400-700 nm).

Table S2 : Computational data for complex **1**

Center Number	Atomic Number	Atomic Type	Coordinates (Angstroms) X	Y	Z
1	23	0	1.265690	0.045431	1.307920
2	17	0	0.390564	-1.736927	2.675907
3	17	0	0.266037	1.844801	2.570989
4	7	0	-0.752608	-0.047880	0.122354
5	7	0	1.595750	1.389356	-0.280580
6	7	0	1.693545	-1.353329	-0.209185
7	6	0	0.572986	1.668031	-1.124925
8	6	0	-0.795482	1.189633	-0.713739
9	1	0	-1.237242	1.980582	-0.096311
10	1	0	-1.438855	1.058210	-1.592079
11	6	0	2.837432	1.875367	-0.531509
12	1	0	3.608382	1.610468	0.181479
13	6	0	0.705238	-1.704209	-1.067249
14	6	0	-0.697949	-1.294465	-0.698834
15	1	0	-1.322105	-1.213901	-1.596974
16	1	0	-1.108101	-2.103662	-0.083600
17	6	0	2.961949	-1.788329	-0.418178
18	1	0	3.703143	-1.466430	0.302827
19	6	0	-3.297070	-0.077654	0.256113
20	6	0	-1.978831	-0.087756	1.013397
21	1	0	-1.908570	0.777439	1.676225
22	1	0	-1.886567	-0.986733	1.626263
23	6	0	0.773221	2.444410	-2.271870
24	1	0	-0.059794	2.645808	-2.934757
25	6	0	0.969138	-2.497770	-2.189552
26	1	0	0.162286	-2.754172	-2.865589
27	6	0	2.274651	-2.951885	-2.412948
28	1	0	2.500301	-3.567260	-3.275921
29	6	0	-3.890419	-1.278525	-0.179899
30	1	0	-3.410032	-2.228907	0.037901
31	6	0	-3.967933	1.135138	0.005184
32	1	0	-3.545106	2.069264	0.367007
33	6	0	-5.761565	-0.050157	-1.131853
34	1	0	-6.707043	-0.039578	-1.663833
35	6	0	-5.189348	1.152290	-0.685602
36	1	0	-5.694219	2.095351	-0.866711
37	6	0	-5.110700	-1.267554	-0.872692
38	1	0	-5.554825	-2.201899	-1.199713
39	8	0	2.772318	0.114953	1.807789

40	6	0	3.099557	2.669191	-1.648855
41	1	0	4.100146	3.045191	-1.817334
42	6	0	3.286964	-2.598786	-1.507212
43	1	0	4.307344	-2.932530	-1.642492
44	6	0	2.050727	2.953101	-2.537480
45	1	0	2.227023	3.556892	-3.419968

Table S3 : Computational data for complex **2**

Center Number	Atomic Number	Atomic Type	Coordinates (Angstroms) X	Y	Z
1	5	0	-6.839758	0.594765	-0.107048
2	9	0	-7.796335	0.823501	0.955211
3	9	0	-7.559424	0.838184	-1.345589
4	7	0	-5.658396	1.545592	0.015245
5	6	0	-4.302078	1.148604	0.049689
6	6	0	-3.500795	2.337300	0.084131
7	6	0	-4.397978	3.416527	0.080027
8	6	0	-5.717878	2.905541	0.039121
9	6	0	-3.958143	-0.213110	0.036834
10	6	0	-4.954898	-1.198664	-0.015710
11	7	0	-6.320578	-0.834615	-0.065962
12	6	0	-7.076152	-1.966459	-0.068345
13	6	0	-6.212715	-3.088974	-0.019312
14	6	0	-4.887412	-2.631214	0.017188
15	1	0	-4.139436	4.465039	0.098341
16	1	0	-6.535834	-4.119439	-0.006707
17	6	0	-2.522626	-0.605633	0.095747
18	6	0	-1.842043	-0.589822	1.323275
19	6	0	-1.845770	-0.994132	-1.070938
20	6	0	-0.487786	-0.943951	1.378470
21	1	0	-2.370803	-0.307538	2.228536
22	6	0	-0.489367	-1.338788	-1.009614
23	1	0	-2.375972	-1.026298	-2.017796
24	6	0	0.212309	-1.302817	0.210581
25	1	0	0.020225	-0.952495	2.338775
26	1	0	0.020298	-1.653015	-1.916382
27	6	0	1.687007	-1.665919	0.264627
28	7	0	2.614771	-0.481846	0.081670
29	1	0	1.943746	-2.117767	1.224970
30	1	0	1.938478	-2.382131	-0.520322
31	23	0	4.885761	-1.066709	0.111778
32	6	0	2.418943	0.481610	1.206160
33	6	0	2.369029	0.136981	-1.255430
34	17	0	4.305498	-2.813899	-1.449814
35	17	0	4.385306	-2.324050	2.110287
36	7	0	4.848199	0.704136	1.252066
37	7	0	4.791405	0.334696	-1.459272
38	8	0	6.472017	-1.142662	0.086231
39	6	0	3.654014	1.291041	1.509974
40	1	0	2.203095	-0.110019	2.103382
41	1	0	1.560122	1.140722	1.027850
42	6	0	3.582650	0.835036	-1.813572
43	1	0	1.509211	0.817719	-1.233050
44	1	0	2.128957	-0.677885	-1.948198
45	6	0	6.001954	1.325015	1.604749
46	6	0	5.924306	0.839161	-2.009793
47	6	0	3.583228	2.547320	2.122566
48	6	0	3.475250	1.885421	-2.731975
49	1	0	6.919466	0.802366	1.364983
50	6	0	5.999187	2.569339	2.236566
51	1	0	6.855860	0.396846	-1.678855
52	6	0	5.884399	1.874293	-2.945315
53	1	0	2.616114	2.999838	2.306873
54	6	0	4.768951	3.194818	2.491880
55	1	0	2.497479	2.274400	-2.989845
56	6	0	4.639302	2.411612	-3.306466
57	1	0	6.937135	3.034922	2.509330
58	1	0	6.806680	2.251346	-3.367586
59	1	0	4.734880	4.167645	2.968088
60	1	0	4.577426	3.225321	-4.019433
61	6	0	-2.002786	2.461346	0.112846
62	1	0	-1.594480	2.150101	1.080472

63	1	0	-1.526740	1.837688	-0.651093
64	1	0	-1.714938	3.501940	-0.058204
65	6	0	-3.670610	-3.510199	0.083272
66	1	0	-3.105906	-3.481029	-0.854446
67	1	0	-2.986564	-3.199767	0.879385
68	1	0	-3.970186	-4.545141	0.266963
69	6	0	-8.570460	-1.943628	-0.094166
70	1	0	-8.964885	-1.630963	0.878837
71	1	0	-8.939471	-1.231909	-0.838419
72	1	0	-8.958195	-2.937637	-0.323716
73	6	0	-7.007902	3.660779	0.032109
74	1	0	-7.673253	3.293650	-0.755107
75	1	0	-7.530880	3.534737	0.986155
76	1	0	-6.819909	4.724157	-0.125192