Supplementary Information

Biological Evaluation of Dinuclear Copper complex/Dichloroacetic acid Cocrystal against Human Breast Cancer: Design, Synthesis, Characterization, DFT studies and cytotoxicity Assays.

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Experimental

Infra-Red and Elemental Analysis data.
H_2valdien Yield = 93%. IR (KBr, cm^{-1}): 3428, 2936, 2935, 1656, 1479, 1376, 1361, 1272, 1157, 1037, 922, 829, 775, 735, 665, 470. Ana. Calcd for $C_{20}H_{25}N_3O_4$: C, 64.67; H, 6.78; N, 11.31. Found: C, 64.56; H, 6.53; N, 11.27.

Cocrystal $[Cu_2(valdien)_2](Cl_2CHCOOH)_2$ (1) Yield = 65-70%. IR (KBr, cm^{-1}): 3428, 3053, 2999, 2953, 2834, 1655, 1617, 1551, 1471, 1452, 1340, 1317, 1246, 1227, 1171, 1083, 978, 862, 819, 734, 711, 628, 568, 530, 472, 443. Ana. Calcd for $C_{44}H_{50}Cl_4Cu_2N_6O_{12}$: C, 47.03; H, 4.48; N, 7.48. Found: C, 63.95; H, 4.31; N, 7.42.

Table S1. Selected bond lengths (Å) **1.**

Bond lengths	(Å)
N1 Cu1	1.973(5)
N2 Cu1	1.986(5)
N5 Cu2	1.981(5)
N6 Cu2	1.973(5)
O1 Cu1	1.892(4)
O3 Cu1	1.875(4)
O5 Cu2	1.879(4)
O7 Cu2	1.904(4)

Table S2. Selected bond angles of **1**

Bond Angle	[deg]
C33 N1 Cu1	123.5(5)
C32 N1 Cu1	120.2(4)
C8 N2 C9	117.4(5)
C8 N2 Cu1	123.1(5)
C9 N2 Cu1	119.3(4)
C28 N5 Cu2	123.2(5)
C29 N5 Cu2	120.0(4)
C13 N6 Cu2	124.1(4)
C12 N6 Cu2	119.6(4)
C1 O1 Cu1	126.6(4)
C39 O3 Cu1	128.8(4)
C19 O5 Cu2	129.2(4)
C21 O7 Cu2	126.9(4)
O3 Cu1 O1	160.2(2)
O3 Cu1 N1	93.6(2)
O1 Cu1 N1	90.9(2)
O3 Cu1 N2	90.2(2)
O1 Cu1 N2	92.7(2)
N1 Cu1 N2	158.6(2)
O5 Cu2 O7	161.0(2)
O5 Cu2 N6	92.9(2)
O7 Cu2 N6	91.2(2)
O5 Cu2 N5	90.4(2)
O7 Cu2 N5	92.9(2)
N6 Cu2 N5	157.2(2)

Table S3. Hydrogen bond metrics for **1**

$D-H\cdots A$	**H···A**	**D···A**	$\angle\, D-H\cdots A$	**Symmetry operation**
N(3)-H(3A)···O(1)	2.25	2.84 (11)	143	
N(3)-H(3A)···O(2)	2.37	2.97 (12)	145	
N(4)-H(4A)···O(7)	2.28	2.90 (11)	142	
N(4)-H(4A)···O(8)	2.35	3.01 (12)	148	
C(7)-H(7B)···O(10)	2.35	3.21 (13)	150	1+x, 1+y, z
C(10)-H(10B)···O(3)	2.30	2.92 (11)	121	
C(10)-H(10B)···N(6)	2.59	3.23 (13)	124	
C(11)-H(11B)···O(9)	2.55	3.34 (13)	138	x,1+y,z
C(12)-H(12A)···Cl(3)	2.80	3.52 (14)	131	x,1+y,z
C(12)-H(12A)···Cl(31)	2.70	3.49 (14)	140	x,1+y,z
C(12)-H(12A)···O(7)	2.36	2.87 (11)	112	
C(30)-H(30B)···O(5)	2.30	2.94 (12)	123	
C(30)-H(30B)···N(1)	2.62	3.22 (13)	121	
C(32)-H(32A)···O(1)	2.41	2.88 (11)	109	

C(33)-H(33)···O(12)	2.54	3.00 (12)	111	1-x,1-y,1-z
C(36)-H(36)···Cl(3)	2.82	3.62 (14)	145	
C(36)-H(36)···Cl(31)	2.79	3.65 (14)	155	
C(41)-H(41)···O(11)	2.52	3.38 (13)	148	-x,-y,1-z
C(43)-H(43)···O(9)	2.31	3.17 (12)	157	-x,-y,-z

Table. S4 B3LYP/D3BJ/DFT optimized structure coordinates of cocrystal [Cu_2(valdien)$_2$····2Cl_2CHCOOH] **1**.

Cu	5.80945136797031	-3.74032959451941	9.46378262494520
C	3.50012434944886	-4.93082756097047	13.16308183483593
H	3.72995097178418	-4.24271451233371	13.98466388501789
H	2.72525707194949	-5.65034094898156	13.48009388094240
O	5.58907739841315	-3.03723794388200	13.15374384480221
O	5.07802960018949	-5.48827146057194	9.31558282661131
C	8.75889936044614	-3.45654289460391	8.92630001561775
H	8.45316886103504	-2.43875773412858	8.65272621812981
H	9.55889702299328	-3.79387424915593	8.24919046780759
O	8.94544457072296	-7.64450672223729	10.44181536693959
O	3.92288196541539	-1.06932350160767	13.10010040590619
N	2.55201122698096	-4.96345646345054	10.88174020060855
C	9.28619897959351	-3.39067376532461	10.35640002586976
H	10.23066963849065	-2.82894246646210	10.35591386639538
H	8.55903397633794	-2.88836621235355	10.99972675046147
N	9.60195933048389	-4.74753399666454	10.87181255816173
C	5.20375478566368	-9.08446797091200	11.54707011732641
H	4.20225897056034	-9.41666695950337	11.83151255783768
C	7.75085116246651	-8.18300852849605	10.79629596146519
N	4.01037708772086	-2.86330816288351	9.23061247826098
N	8.23375115874514	-3.94855608035745	13.35752931523268
O	6.55964948081475	-2.17334502448629	10.22796131804580
C	1.94821370311426	-4.24583184417026	9.77925835778426
H	1.31794983425321	-3.43418025319933	10.18178325882524
H	1.26367073921501	-4.91875746759005	9.22938751675204
O	7.32093475460640	-6.08289329892702	11.76019397104514
C	5.55642155709796	-6.50717092597519	8.67290816678759
O	8.25263146710735	-0.32311800849917	10.87590781439109
C	6.06607115915869	-9.93523908247655	10.89084114995992
H	5.76083758651844	-10.95274829942995	10.63861466410481
N	4.71769318350736	-5.65391594668462	12.79034820800066
C	2.89823429624031	-3.66007309908484	8.70959122122092
H	3.33141595951136	-4.49617298064514	8.14629815759195
H	2.29000031562626	-3.03764585571247	8.03363657791012
C	6.98781995682784	0.09261524629925	10.60771815363769
C	5.58772662270364	-7.75228222528082	11.85457742162342
N	7.60855730778639	-4.35213317528732	8.81075442709400
C	9.90630057606964	-8.46866840316244	9.80037332690088
H	10.73033931215845	-7.80891462174842	9.50347438870367
H	9.48596192037689	-8.94280444364785	8.89830052858557

H	10.26065035361603	-9.26001345792961	10.48500542479531
C	6.08544695210221	-1.88973883600728	13.45268745232915
C	7.88252511415709	-0.35939457787979	14.16949816815656
H	8.93040658987744	-0.21171463156553	14.44448971548787
C	4.33867242630807	0.73909896329938	9.94859544584628
H	3.31148593568377	0.98484469847529	9.66633175943634
C	8.41193366752438	-2.72351583367157	13.75751047576544
C	7.45297118674669	-1.66266291598381	13.78853120545221
C	2.98495711465916	-4.11246207024753	11.98032410554036
H	2.11796034610295	-3.52550287989209	12.33429957469650
H	3.76699732072993	-3.38280508308750	11.72079962137102
C	9.38801588181578	-4.85349165132828	13.38930759229971
H	9.00107397031962	-5.87739395871747	13.45927784588845
H	10.00069177621048	-4.65947759545136	14.28699941199097
C	6.08980079720533	-0.97032476507014	10.24300549950072
C	5.18881980990968	-0.76126625643942	13.47154659899677
C	6.99978270745525	0.69651988446648	14.19158163032719
H	7.33585232839311	1.69246166068188	14.48722990278965
C	10.31374836845251	-4.75360295837075	12.17072411302454
H	10.94804330894907	-3.85719909542196	12.20330621259810
H	10.99683381082572	-5.61651573763084	12.17539548035233
C	5.21441963444894	1.73275973156089	10.33272206956122
H	4.88725426123430	2.77400657578180	10.37615417129012
C	4.75441294299584	-0.61892623204801	9.88769384383830
C	4.59579919890235	-6.89433779917111	12.44539274072585
C	3.81390391455877	-1.59578372018882	9.40822664376189
C	2.94927780994122	-0.05431290048091	13.08452655819045
H	2.02026538886562	-0.52342355311291	12.73192591665045
H	3.22445027074982	0.76146206815227	12.39524671834290
H	2.78188984898604	0.36826766033987	14.09300744150102
C	9.25279157505424	0.64088214623246	11.09547048169273
H	10.19389748307850	0.08718335863513	11.22097076225652
H	9.05775940100217	1.23152175569615	12.00773337259621
H	9.35143734194748	1.32992248671256	10.23640562872411
C	6.55283679393944	1.40653540911845	10.65187170905538
H	7.24123021446408	2.20121542987554	10.94005646392588
C	6.87958102713353	-7.28400095011261	11.49479966863150
Cu	6.46137990299184	-4.66846529429442	12.73316625264302
C	7.81919764041885	-5.51640793502723	8.27755067826513
C	7.28304538398879	-7.75226857025782	7.43530408550302
H	8.29448980033376	-7.78252916607319	7.02353911465847
C	6.86640126693804	-6.58043623946004	8.12155619624362
C	6.43415002737679	-8.82850006721548	7.28770804792607
H	6.76290258129493	-9.72396315770844	6.75645997719545
C	5.12856758471383	-8.77372470529623	7.82915627736979
H	4.46642250874427	-9.63322083723059	7.71578280261325
C	7.34676923585146	-9.47976583370433	10.51185654828368
H	8.01359008339246	-10.15165695522313	9.97296216191883
O	3.46930080786821	-7.47814519943722	9.06508284408692
C	5.64218232858705	0.49281387018929	13.83765400628986
H	4.95953606836233	1.34311583802448	13.83920703746982
C	4.69542753300284	-7.64394670427586	8.50335601955602
C	2.53875791695105	-8.52739740509385	8.99699161119054
H	1.64509744902785	-8.17878935135434	9.53145251891173

H	2.92469252278352	-9.44154916027319	9.48245859001212
H	2.26731546288773	-8.76868665843697	7.95278394260934
H	8.74920657476490	-5.33962373501770	10.96546541549440
H	3.32909731188612	-5.51762489182511	10.51733184340471
Cl	0.12173398660264	-4.28688780912294	4.47420536168197
Cl	0.23409469471137	-6.28480319868993	6.65821182120599
C	-0.44489909076900	-3.64835310144081	7.04725319955909
C	0.46868413371341	-4.55746496923724	6.20723799834783
O	-1.71848002352293	-4.02854303490787	7.14910206888291
H	-1.83908948331805	-4.91148843929520	6.75953314354667
O	-0.02276766505148	-2.64105544597878	7.54272281685199
Cl	13.65054525246859	-7.45761310670337	8.59683445703757
C	13.38487728889347	-5.70416385671029	8.27528978091215
O	12.55688569603328	-4.28384858172835	9.98463803860827
C	12.24954238512597	-5.16321723090205	9.17583912606324
Cl	13.00943086946168	-5.40740073776863	6.54433166764866
O	11.10706317595752	-5.69432251168748	8.99983734052177
H	10.24075494031970	-5.21901747152039	10.10830563112432
H	14.32353965224022	-5.19066829863134	8.50201267667966
H	1.51589426899399	-4.30524383611546	6.39370818870736
H	3.60468099630404	-7.35800323879624	12.57968846517010
H	8.83780685525988	-5.73570671906623	7.91939093306990
H	2.82095430431734	-1.19411397816315	9.14055256153858
H	9.42136130563899	-2.44494020465316	14.10680149166605

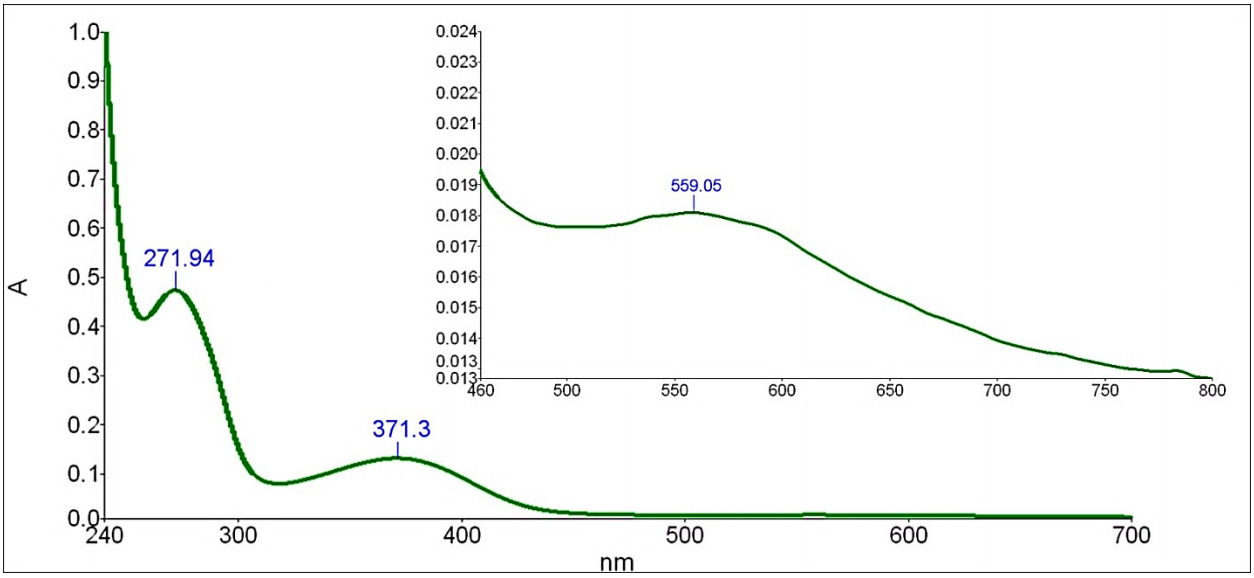


Fig. S1 UV-vis spectra in MeOH (1×10^{-4} M).

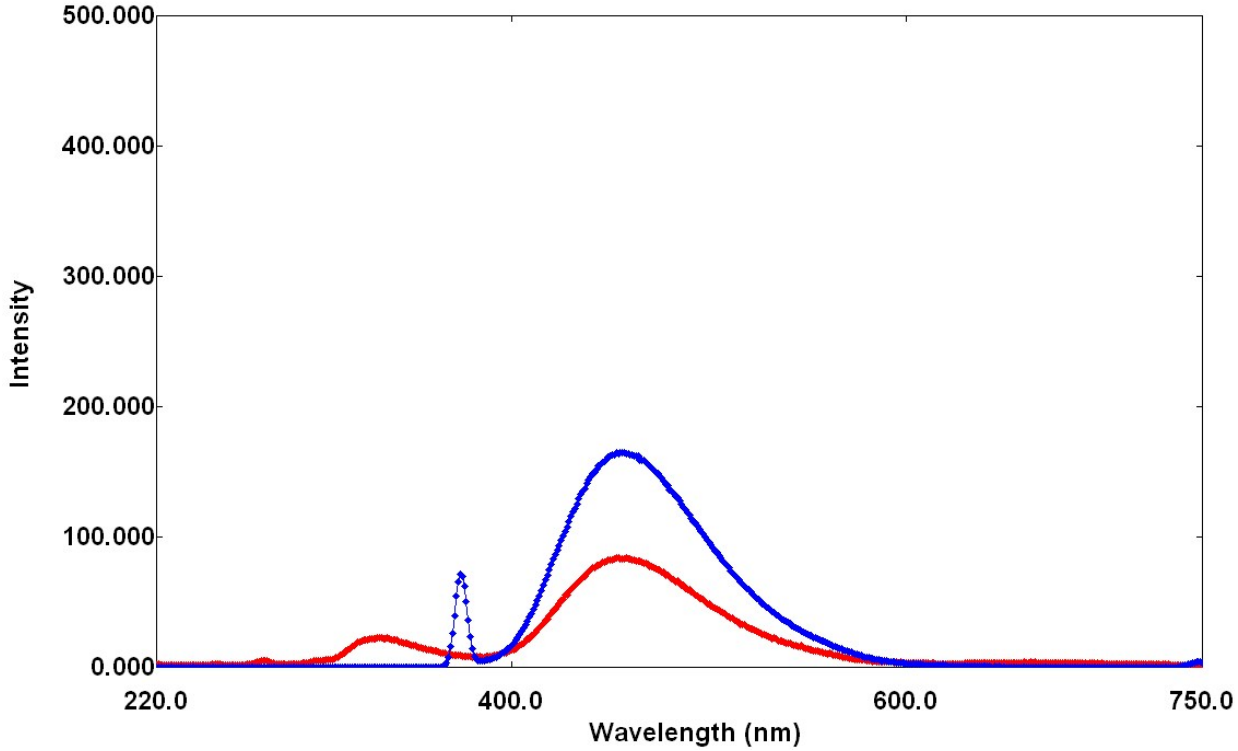


Fig. S2 Fluorescence spectra in MeOH (1×10^{-4} M).

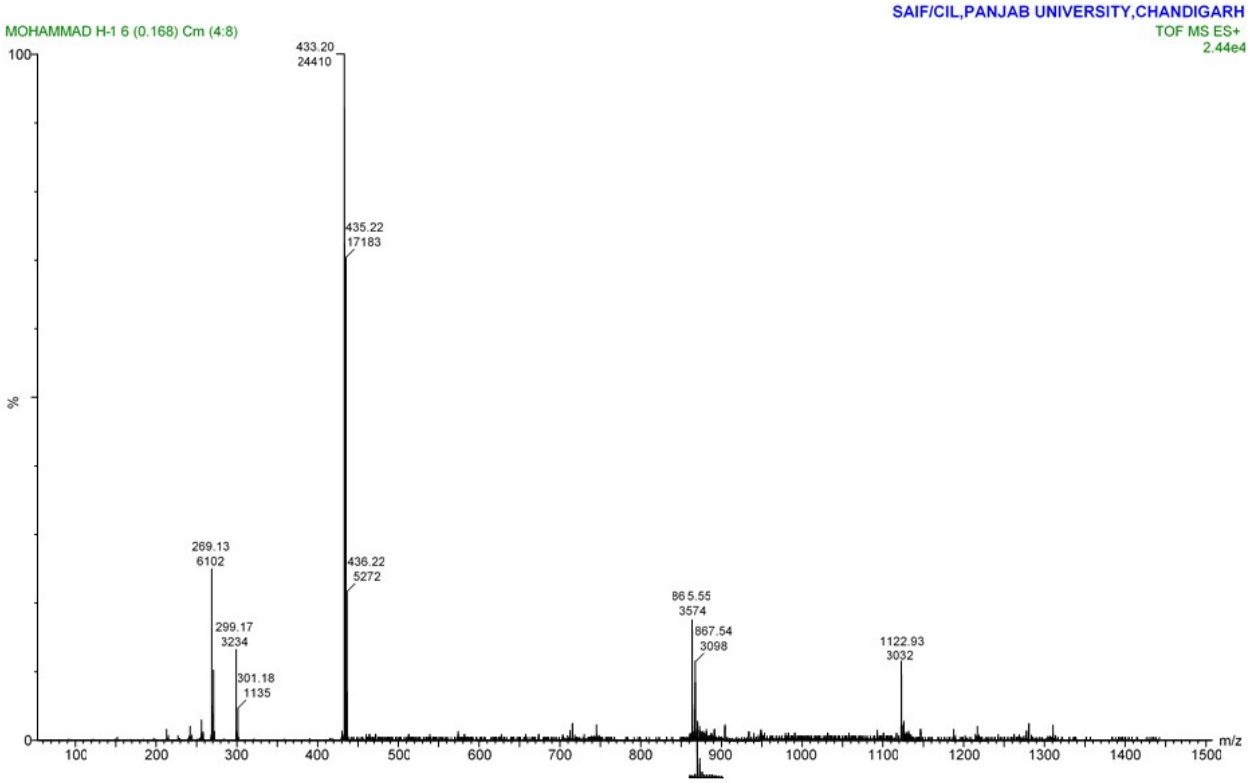


Fig. S3 ESI-Mass spectra of $[Cu_2(valdien)_2\cdots2Cl_2CHCOOH]$.

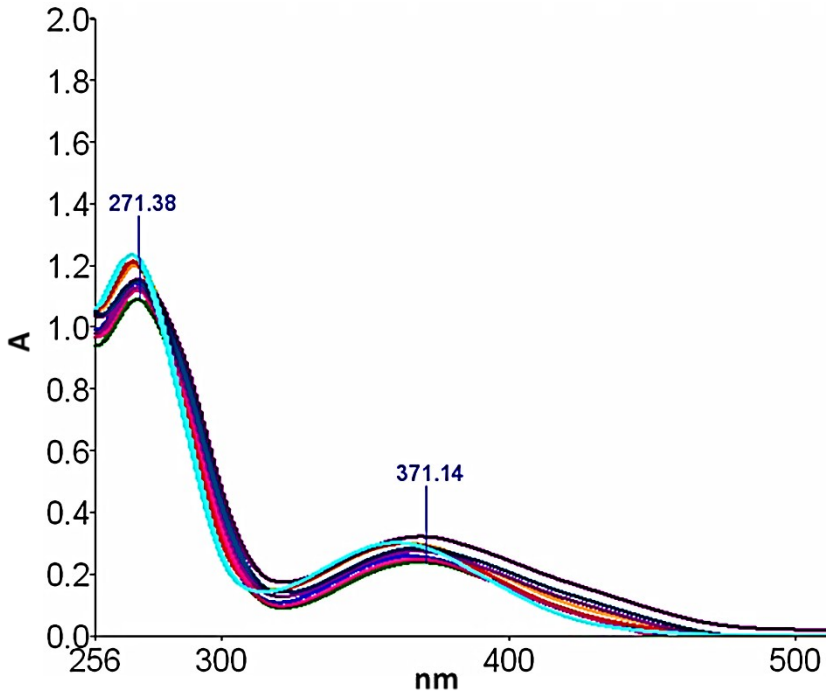


Fig. S4 Time-dependent stability studies of $[Cu_2(valdien)_2\cdots 2Cl_2CHCOOH]$ in Tris-HCl buffer under physiological conditions (pH 7.3 & T 310 K) monitored by UV−vis absorption spectra.

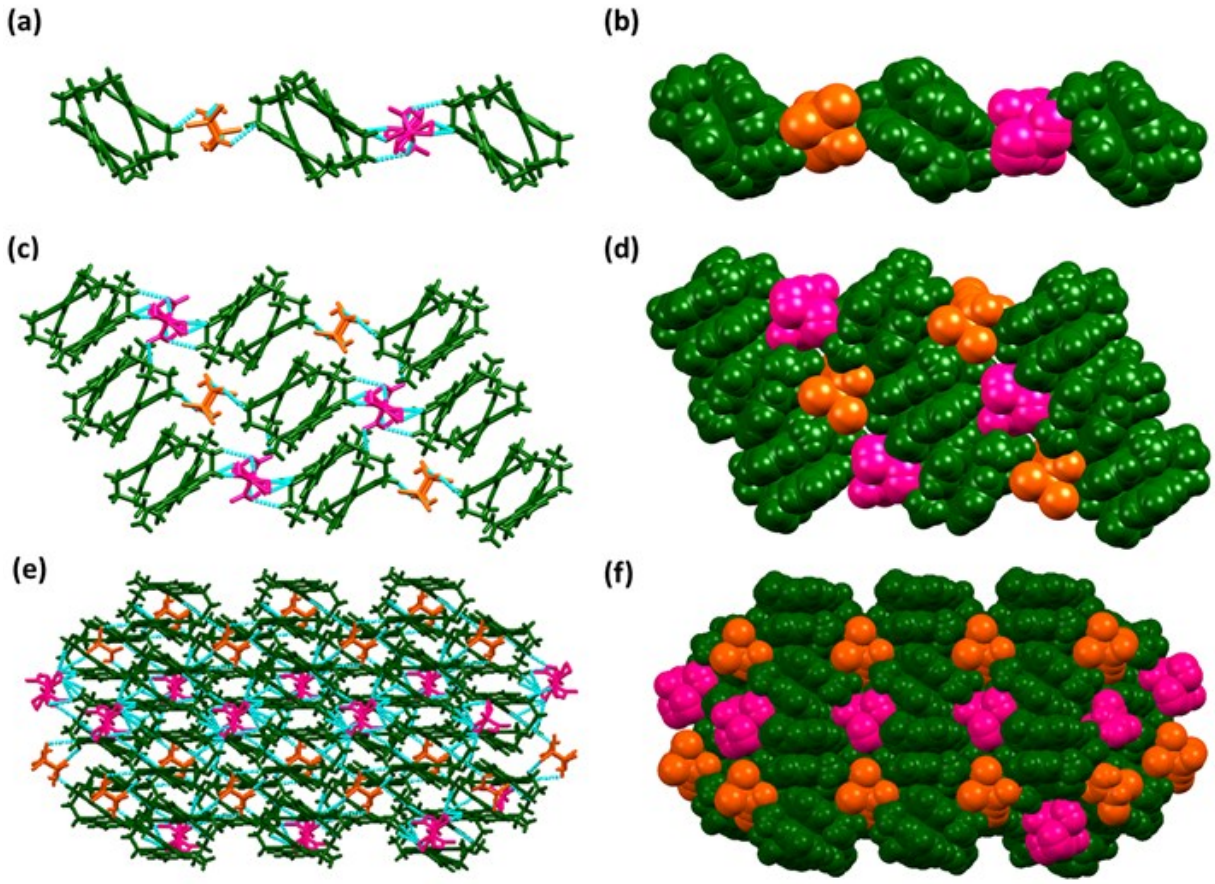


Fig. S5 Supramolecular structure of cocrystal [Cu_2(valdien)$_2$$\cdots$$2Cl_2CHCOOH$] **1**. (a) 1D-chain resulted due to C-H$\cdots$O and C-H$\cdots$Cl hydrogen bonds. Color codes: symmetry equivalent. (b) Space-filled model 1D chain. (c) 2D-sheet (d) Space-filled model of 2D-sheet. (e) 3D-superamolecular structure (f) Space-filled model of the 3D-superamolecular motif.

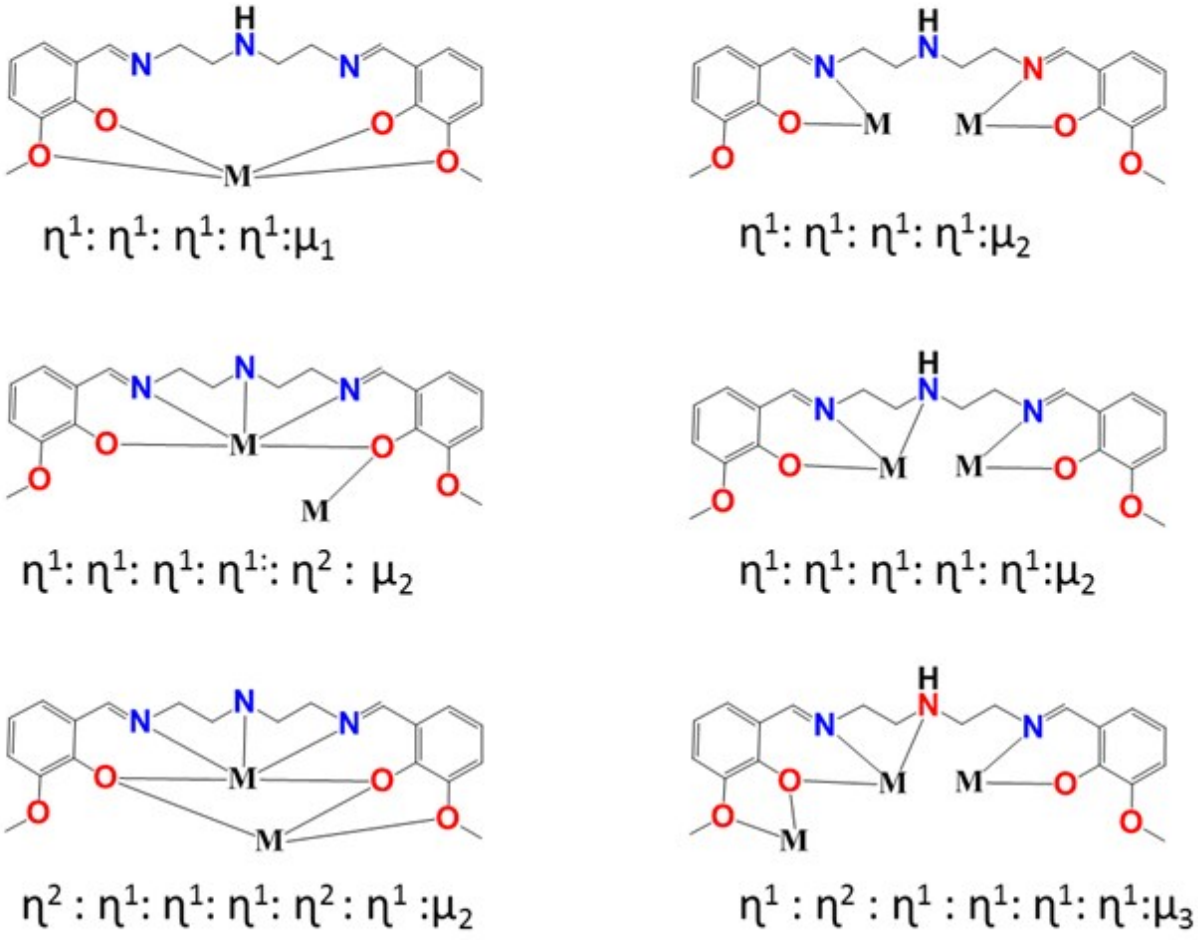


Fig. S6 Various coordination modes of H_2valdien ligand with metal ions.

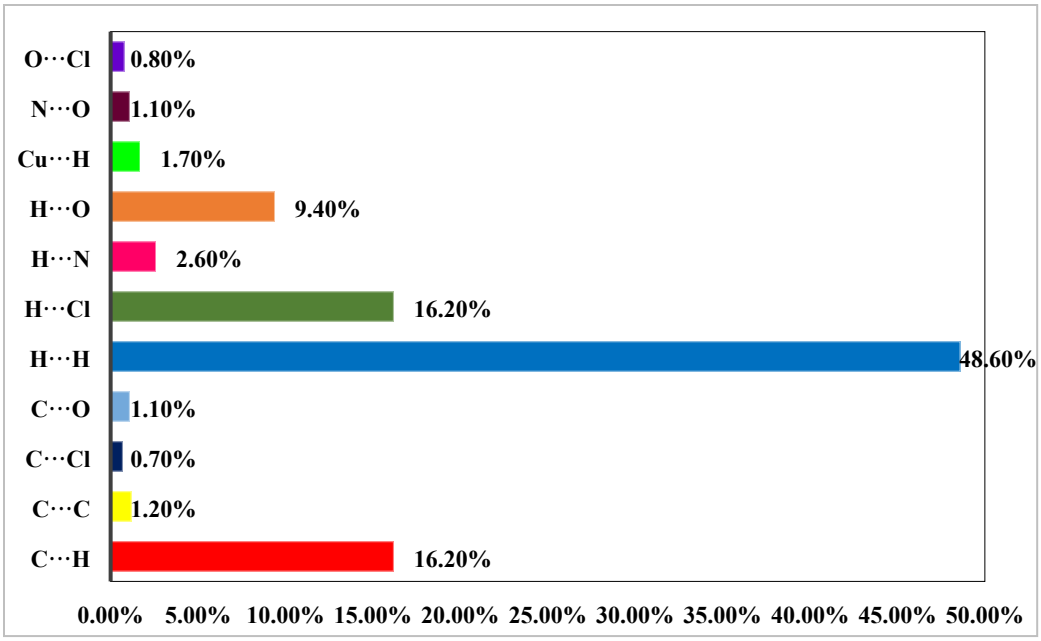
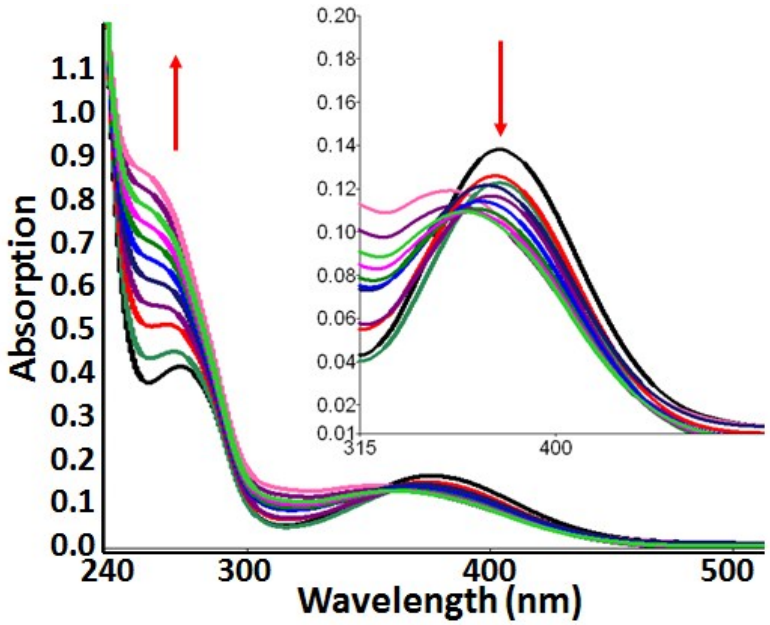
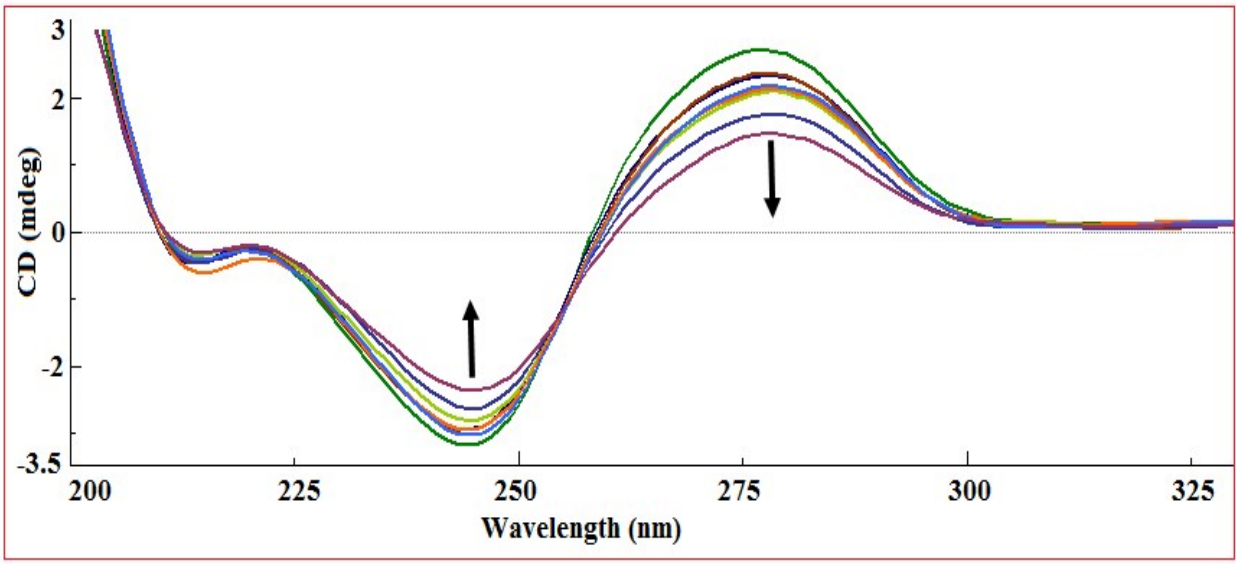


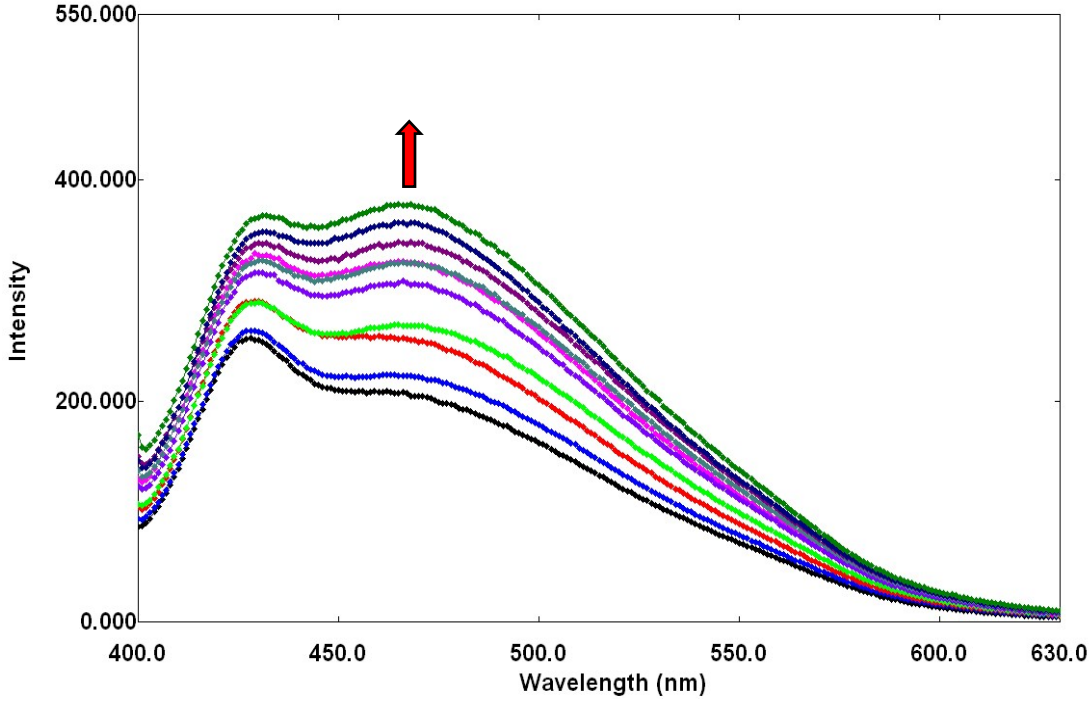
Fig. S7 The relative contribution of various intermolecular contacts to the Hirshfeld surface area in $[Cu_2(valdien)_2\cdots 2Cl_2CHCOOH]$ **1**.



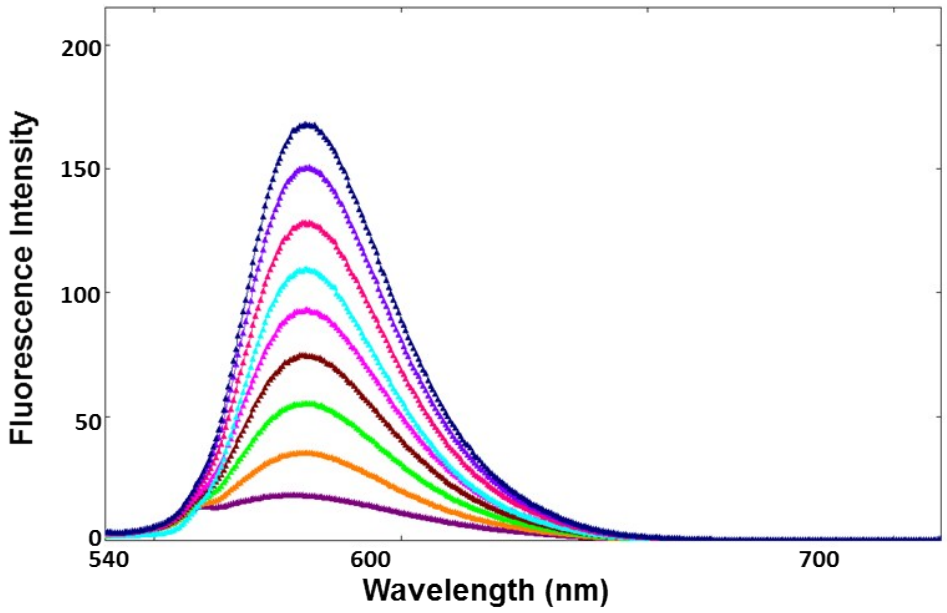
(a)


CD (mdeg)
3
2
0
-2
-3.5
200
225
250
275
300
325
Wavelength (nm)

(b)


Intensity
550.000
400.000
200.000
0.000
400.0
450.0
500.0
550.0
600.0
630.0
Wavelength (nm)

(c)



(d)

Fig. S8 (a) UV−vis spectra of $[Cu_2(valdien)_2\cdots 2Cl_2CHCOOH]$, in Tris−HCl buffer at pH 7.2 upon addition CT−DNA, [DNA] = 0.00–1.8 x 10^{-4} M, [Complex **1**] = 1.0 x 10^{-4} M. Arrow indicates change in absorbance with increasing concentration of CT−DNA. (b) Circular dichroism spectrum of $[Cu_2(valdien)_2\cdots 2Cl_2CHCOOH]$ with the CT-DNA at an increasing ratio (R) of [**1**]/[DNA]. (c) Emission spectra of $[Cu_2(valdien)_2\cdots 2Cl_2CHCOOH]$ in Tris–HCl buffer (pH 7.3) in the presence and absence of CT DNA at room temperature, [DNA] = 0.00–1.8 x 10^{-4} M, $[[Cu_2(valdien)_2\cdots 2Cl_2CHCOOH]]$ = 1.0 x 10^{-4} M. Arrow shows change in intensity with increasing concentration of DNA. (d) Emission quenching spectra of CT DNA bound ethidium bromide in the presence of $[Cu_2(valdien)_2\cdots 2Cl_2CHCOOH]$, in buffer 5 mM Tris–HCl/50 mM NaCl, pH=7.2 at 25 ºC. Arrow shows change in intensity with increasing concentration of $[Cu_2(valdien)_2\cdots 2Cl_2CHCOOH]$.

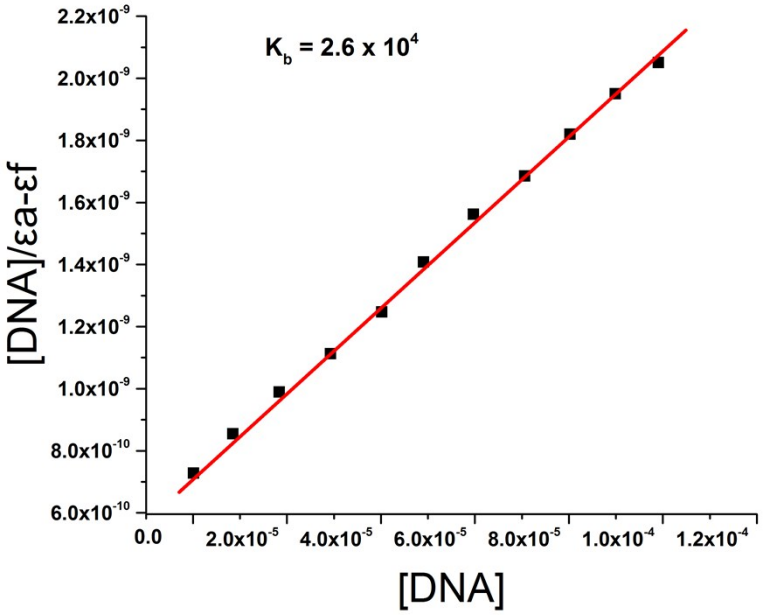


Fig. S9 Plots of [DNA]/ $\varepsilon_a - \varepsilon_f$ (M^2 cm) vs [DNA] for the titration of CT DNA with [Cu_2(valdien)$_2$···2Cl_2CHCOOH] **1**(■), experimental data points; full lines, linear fitting of the data. [Ligand/Complex] = 3.33×10^{-5} M, [DNA] = $(0–8.53)\times10^{-5}$ M. Arrow shows change in intensity with increasing concentration of DNA.

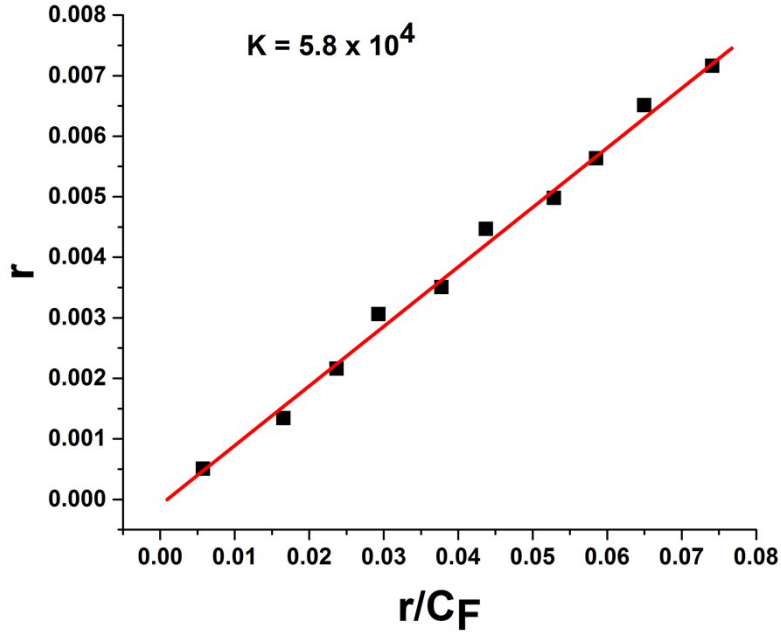


Fig. S10 Fluorescence emission curve of [Cu_2(valdien)$_2$···2Cl_2CHCOOH] **1** in Tris–HCl buffer (pH 7.3) in the presence and absence of CT DNA at room temperature, [DNA] = 0.00–1.8 x 10^{-4} M, [**1**] = 1.0 x 10^{-4} M.

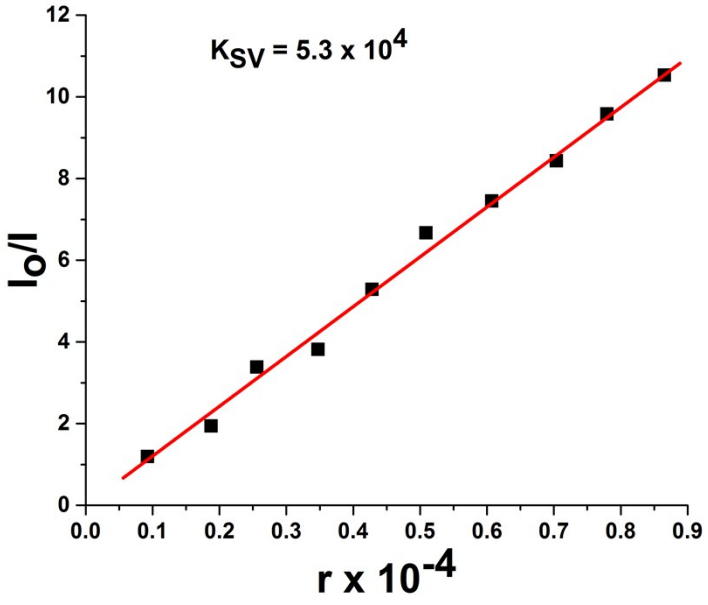


Fig. S11 Emission quenching curve of CT DNA bound ethidium bromide in the presence of $[Cu_2(valdien)_2\cdots 2Cl_2CHCOOH]$ **1**, in buffer 5 mM Tris–HCl/50 mM NaCl, pH=7.2 at 25 ºC.

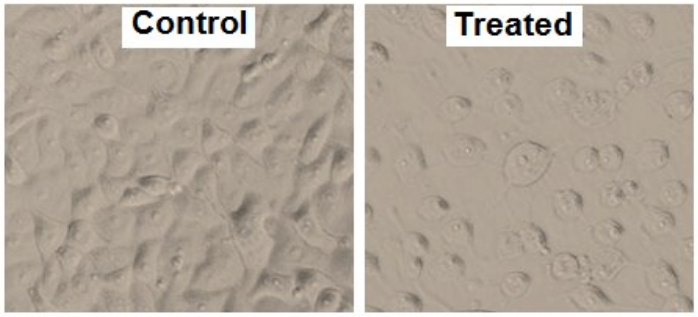


Fig. S12 Morphological changes upon treatment of the complex **1** on MCF 7 breast cancer cell lines.

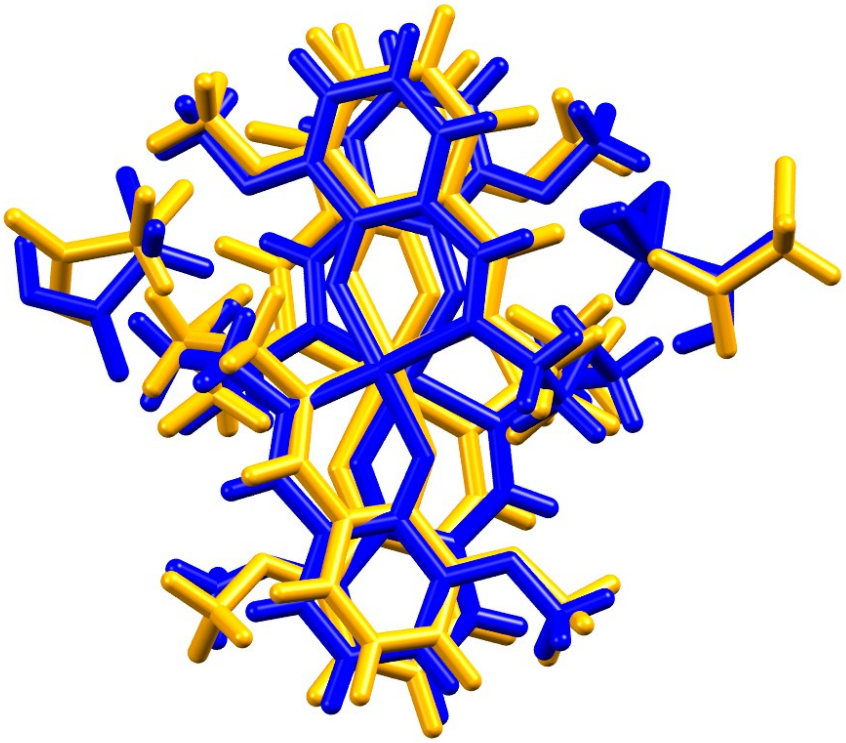


Fig. S13 Single crystal X-ray structure of cocrystal [Cu_2(valdien)$_2$$\cdots$2$Cl_2$CHCOOH] **1** (blue) and gas phase B3LYP/D3BJ/DFT optimized structure of complex **1** (yellow).