Novel copper(II) complexes with fenamates and isonicotinamide: structure and properties, interaction with DNA and serum albumin

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Supplementary material

Albumin binding studies

The extent of the inner-filter effect can be roughly estimated with the following equation:

$$I_{corr} = I_{meas} \times 10^{\frac{\varepsilon(\lambda_{exc})cd}{2}} \times 10^{\frac{\varepsilon(\lambda_{em})cd}{2}} \quad \text{(eq. S1)}$$

where I_{corr} = corrected intensity, I_{meas} = the measured intensity, c = the concentration of the quencher, d = the cuvette (1 cm), $\varepsilon_{(\lambda exc)}$ and $\varepsilon_{(\lambda em)}$ = the ε of the quencher at the excitation and the emission wavelength, respectively, as calculated from the UV-vis spectra of the complexes [1].

The Stern-Volmer and Scatchard equations and graphs have been used in order to study the interaction of a quencher with serum albumins. The Stern-Volmer constant,K_{SV}(in M^{-1}), and the quenching constant, k_q(in $M^{-1}s^{-1}$),may be derived from the Stern-Volmer equation [2]:

$$\frac{Io}{I} = 1 + k_q\tau_0[Q] = 1 + K_{SV}[Q] \quad \text{(eq. S2)}$$

where Io = the initial tryptophan fluorescence intensity of SA, I = the tryptophan fluorescence intensity of SA after the addition of the quencher, k_q= the quenching rate constant, K_{SV}= the Stern-Volmer constant, τ_o = the average fluorescence lifetime of SA (= 10^{-8} s), [Q] = the concentration of

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the quencher, respectively, K_{SV} (in M^{-1}) can be obtained by the slope of the diagram $\frac{Io}{I}$ *versus* [Q], and subsequently k_q (in M^{-1} s^{-1}) may be calculated from equation [2]:

$$K_{SV} = k_q \tau_o \qquad \text{(eq. S3)}$$

From the Scatchard equation [2]:

$$\frac{\Delta I/Io}{[Q]} = nK - K\frac{\Delta I}{Io} \qquad \text{(eq. S4)}$$

the SA-binding constant K (in M^{-1}) may be calculated from the slope in the Scatchard plots $\frac{\Delta I/Io}{[Q]}$ *versus* $\frac{\Delta I}{Io}$ and the number of binding sites per albumin (n) is given by the ratio of y intercept to the slope [2].

DNA binding studies

The DNA-binding constant (K_b, in M^{-1}) can be obtained by monitoring the changes in the absorbance at the corresponding λ_{max} with increasing concentrations of CT DNA and it is given by the ratio of slope to the y intercept in plots [DNA]/(ε_A-ε_f) *versus* [DNA], according to the Wolfe-Shimer equation [3]:

$$\frac{[DNA]}{(\varepsilon_A - \varepsilon_f)} = \frac{[DNA]}{(\varepsilon_b - \varepsilon_f)} + \frac{1}{K_b(\varepsilon_A - \varepsilon_f)} \qquad \text{(eq. S5)}$$

where [DNA] is the concentration of DNA in base pairs, ε_f is the extinction coefficient for the free complex at the corresponding λ_{max}, $\varepsilon_A = A_{obsd}$/[compound] and ε_b is the extinction coefficient for the complex in the fully bound form.

The linear Stern-Volmerequation (eq. S2) has been used in order to study the quenching of EB bound to DNA by the compounds, where I_o and I are the emission intensities of EB-DNA conjugate in theabsence and the presence of the quencher, respectively, [Q] is the concentration of the quencher (i.e. compounds) [2]. The values of the Stern-Volmer constant K_{SV} (M^{-1}) are obtained by the slope of the diagram $\frac{I_0}{I}$ *versus* [Q]. Taking τ_o = 23 ns as the fluorescence lifetime of the EB-DNA system [4], the quenching constants k_q (in $M^{-1}s^{-1}$) of the compounds can be determined according to eq. S3.

References

1 L. Stella, A.L. Capodilupo, M. Bietti, Chem. Commun., 2008, **39**, 4744-4746.

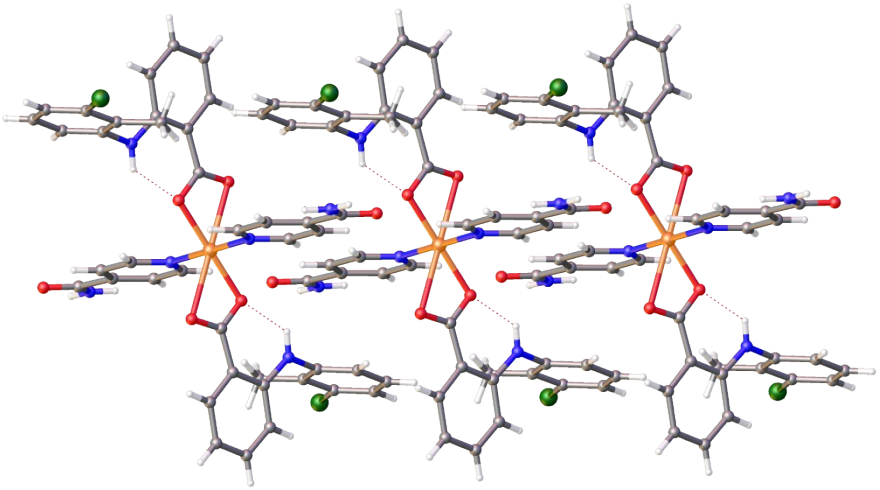
2 J.R. Lakowicz, Principles of Fluorescence Spectroscopy, 3rd Edn, Plenum Press, New York, 2006.

3 A. Wolfe, G. Shimer, T. Meehan., Biochemistry, 1987, **6**, 6392-6396.

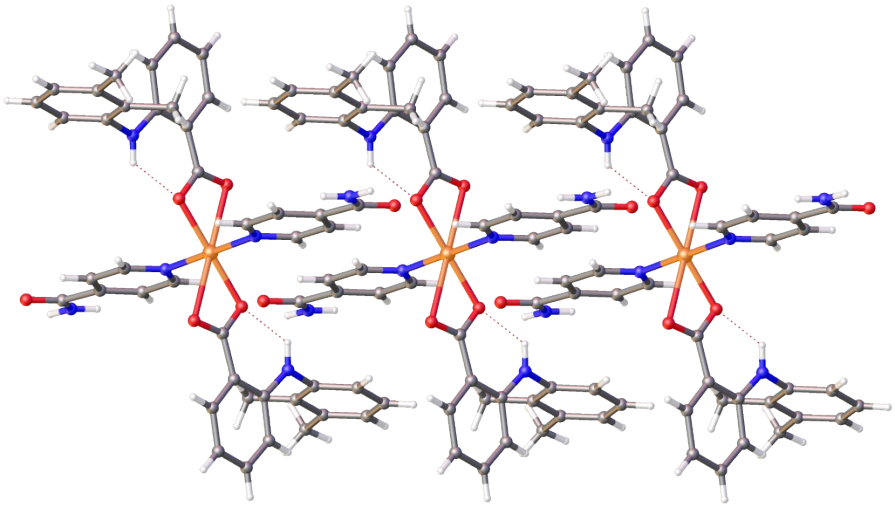
4 D.P. Heller, C.L. Greenstock, Biophys. Chem., 1994, **50**, 305-312.

Table S1. Hydrogen bonds for compounds **1-5.**

D–H···O	d(D–H)/Å	d(H...A)/Å	d(D...A)/Å	D–H...A/°	Symmetry
1					
N2–H2B···O2	0.86	2.13	2.948(3)	160	+*x*, –1+*y*,+*z*
N3–H3···O1	0.86	2.02	2.656(2)	130	+*x*, –1+*y*,+*z*
C4–H4···O2	0.93	2.35	3.224(3)	156	+*x*, –1+*y*,+*z*
2					
N2–H2A···O3	0.86	2.17	3.020(3)	170	1/2–*x*, –1/2+*y*,1/2–*z*
N2–H2B···O2	0.86	2.17	3.015(3)	167	1–*x*, –*y*,1–*z*
N3–H3···O1	0.86	1.91	2.590(3)	135	
C4–H4···O2	0.93	2.40	3.262(3)	155	1–*x*, –*y*,1–*z*
3					
N2–H2B···O2	0.86	2.11	2.934(2)	160	–*x*, –*y*,1–*z*
N3–H3···O1	0.86	2.02	2.659(2)	129	
C4–H4···O2	0.93	2.35	3.255(2)	156	–*x*, –*y*,1–*z*
4					
N2–H2A···O3	0.88	2.05	2.884(3)	158	–*x*, –*y*,–*z*
N2–H2B···O2	0.88	2.04	2.893(3)	164	1–*x*, –*y*,1–*z*
N3–H3···O1	0.88	1.99	2.706(2)	137	
C4–H4···O2	0.95	2.40	3.317(3)	161	1–*x*, –*y*,1–*z*
C19–H19···N4	0.95	2.23	2.860(3)	123	
5					
N2–H2A···O3	0.88	2.00	2.877(2)	158	–*x*, 2–*y*,–*z*
N2–H2B···O2	0.88	2.22	3.081(2)	164	1–*x*, 2–*y*,1–*z*
N3–H3···O2	0.88	1.94	2.679(2)	137	
C4–H4···O2	0.95	2.34	3.284(2)	171	1–*x*, 2–*y*,1–*z*
C19–H19···N4	0.95	2.32	2.927(2)	121	
C1–H1···F1	0.95	2.67	3.288(2)	123	+*x*, +*y*,–1+*z*
C1–H1···F2	0.95	2.66	3.340(2)	129	+*x*, +*y*,–1+*z*
C2–H2···F2	0.95	2.69	3.289(2)	122	+*x*, +*y*,–1+*z*
C13–H13···F2	0.95	2.67	3.376(2)	132	+*x*, +*y*,–1+*z*

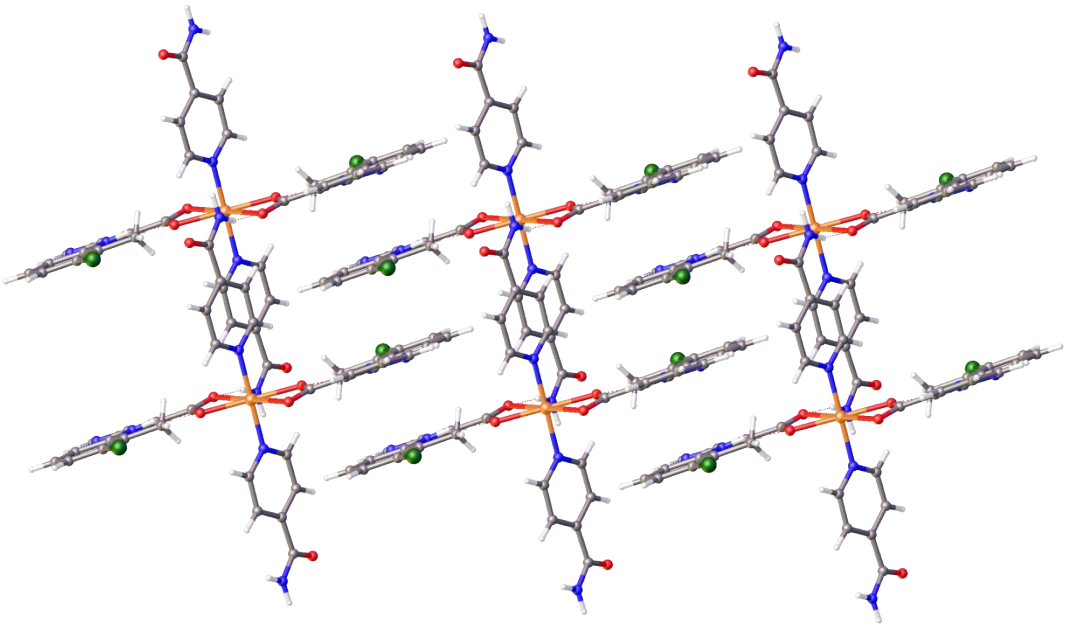


(1)

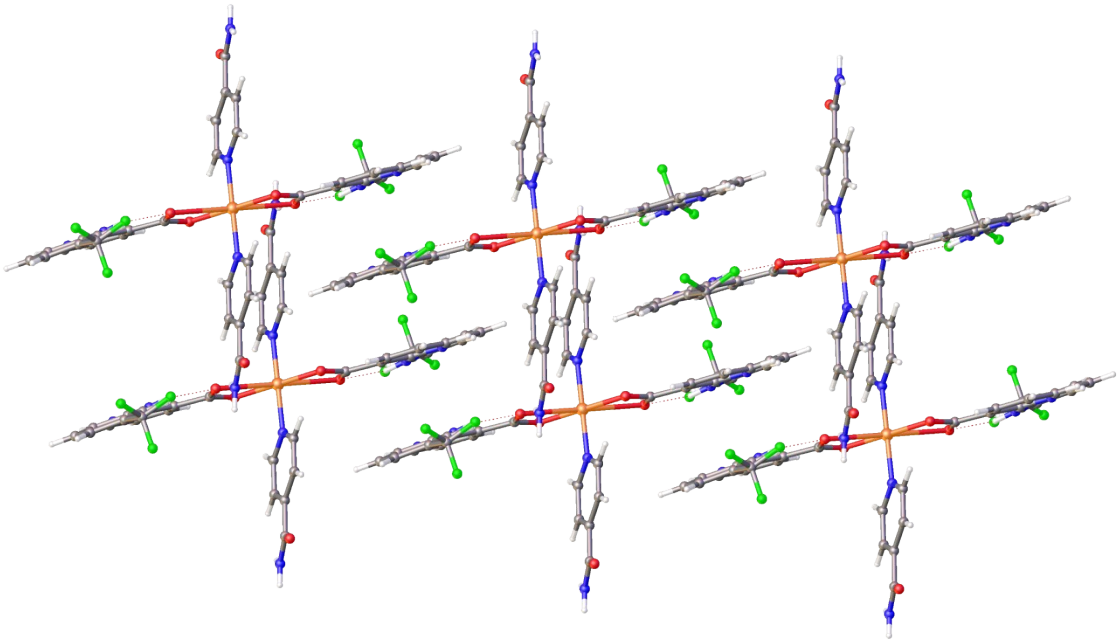


(3)

Figure S1. The π-π stacking interaction between two isonicotinamide ligands in crystal structures of **1** and **3**.



(4)



(5)

FigureS2. The π-π stacking interaction between two fenamateligands in crystal structures of **4** and **5**.

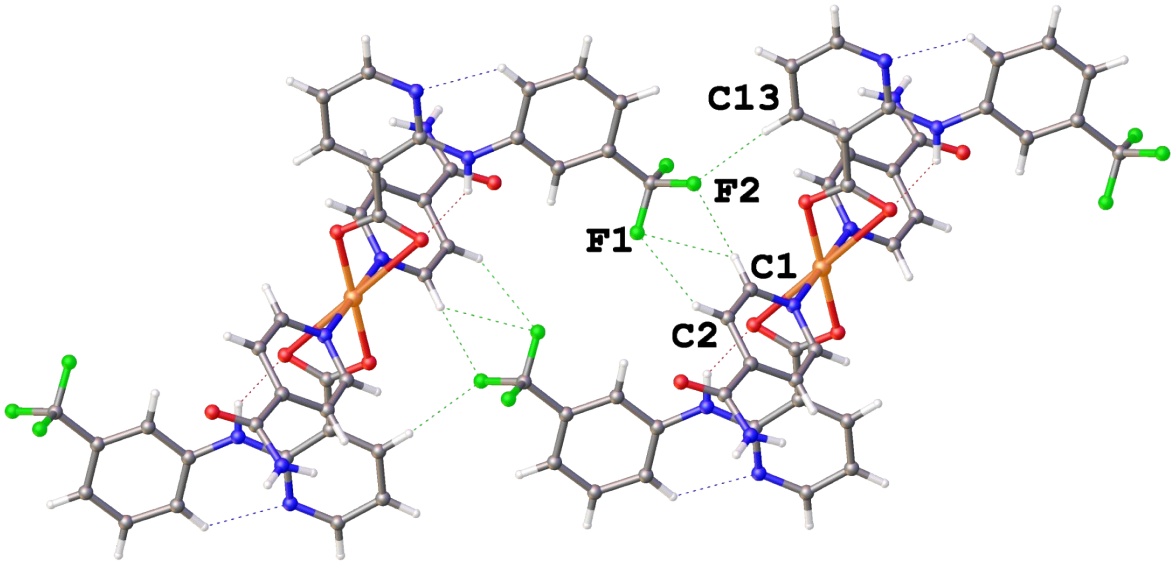


Figure S3. The C–H···F hydrogen bonding interaction in crystal structure of **5**.

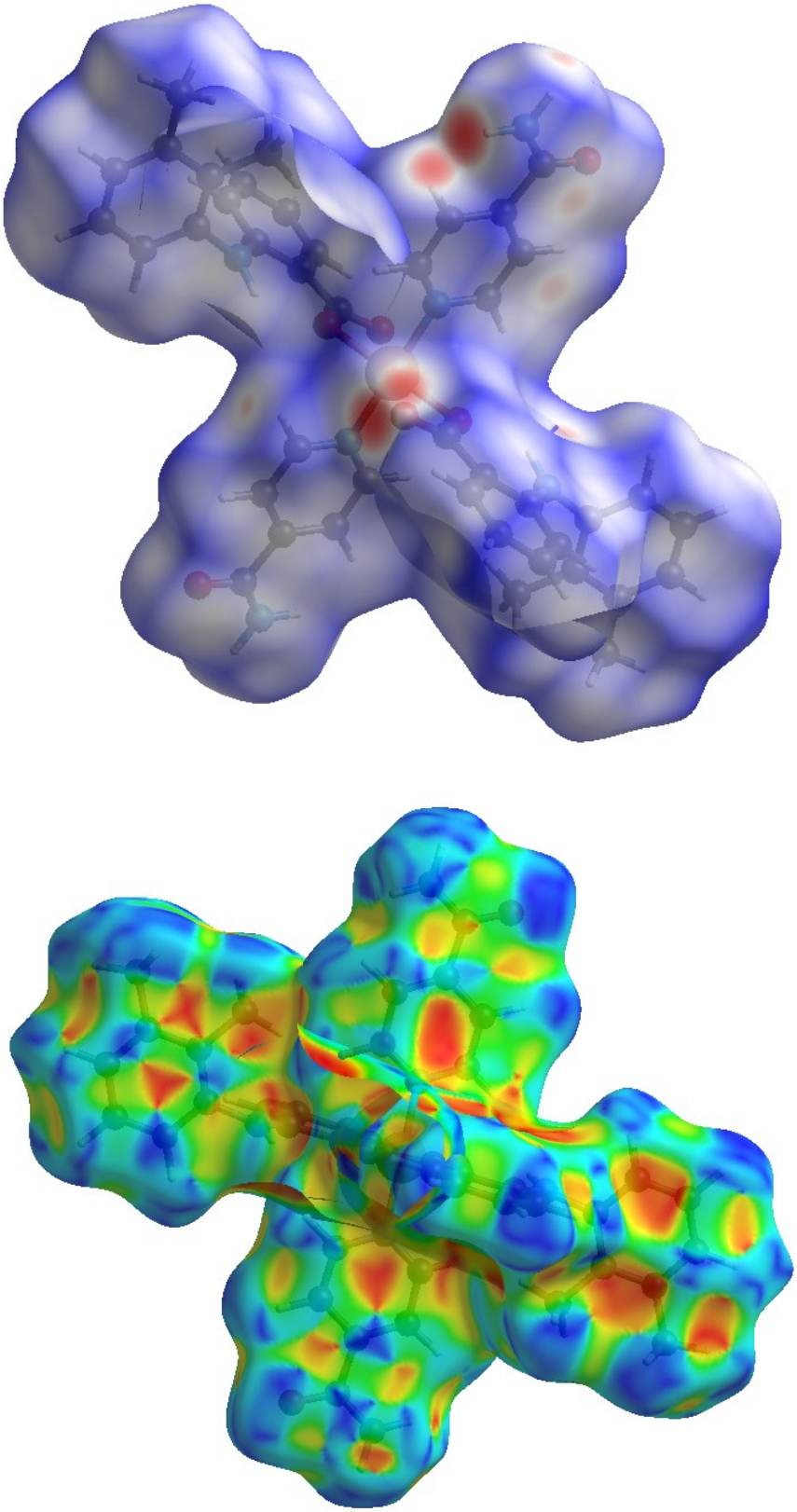


Figure S4. View of the three-dimensional Hirshfeld surface of **3** plotted over d_{norm} in the range -0.4902 to 1.2118 a.u. (top) and shape-index (bottom).

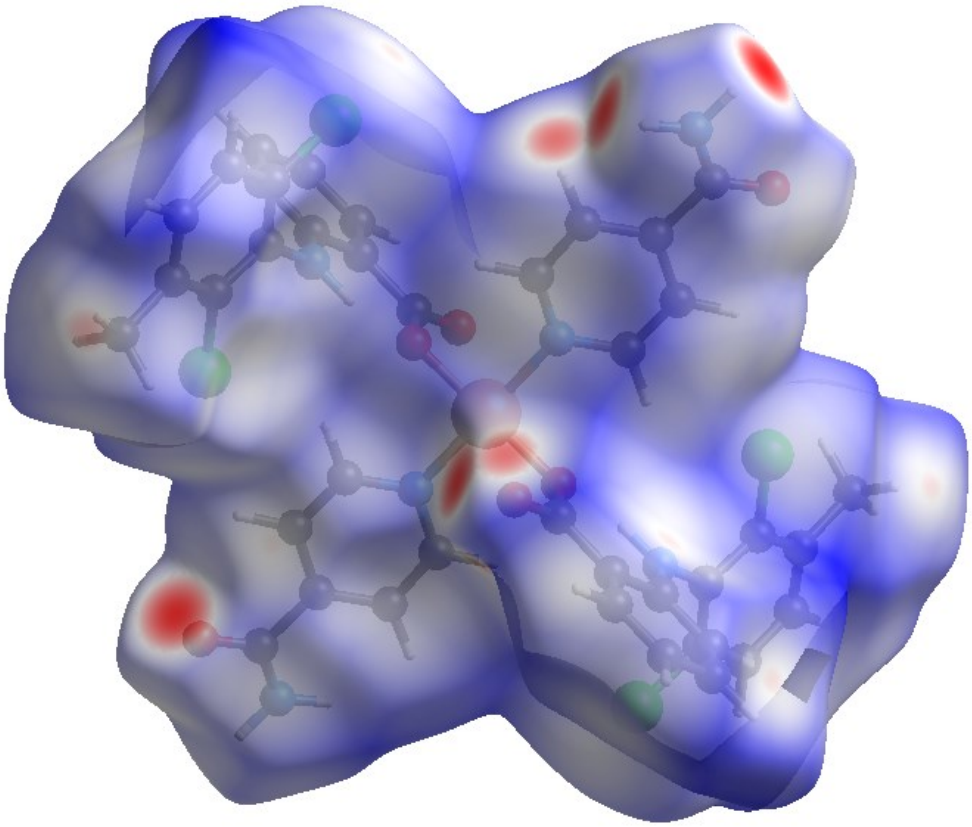


Figure S5. View of the three-dimensional Hirshfeld surface of **2** plotted over d_{norm} in the range -0.4498 to 1.5102 a.u..

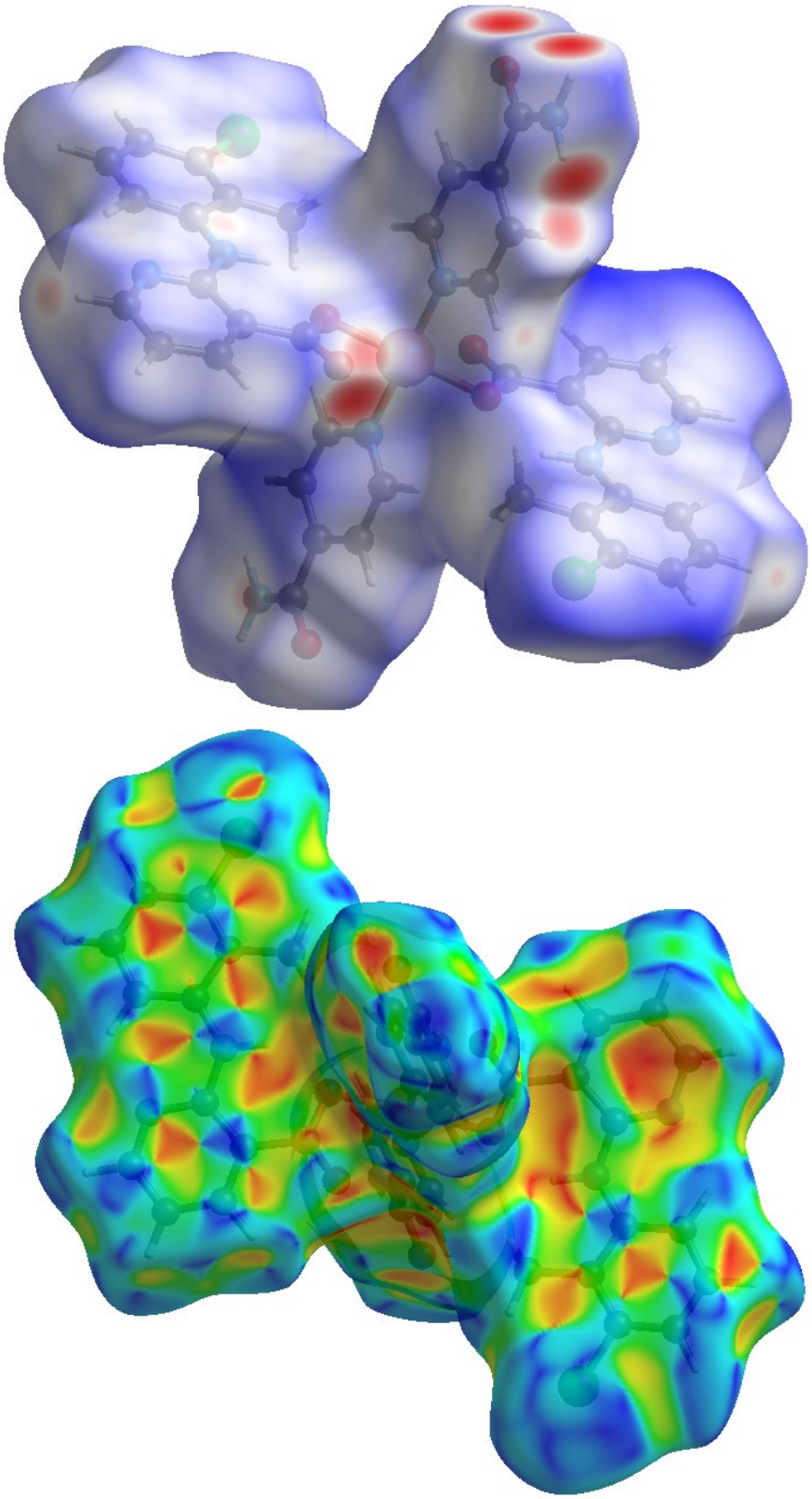


Figure S6. View of the three-dimensional Hirshfeld surface of **4** plotted over d_{norm} in the range -0.5379 to 1.6017a.u. (top) and shape-index (bottom).

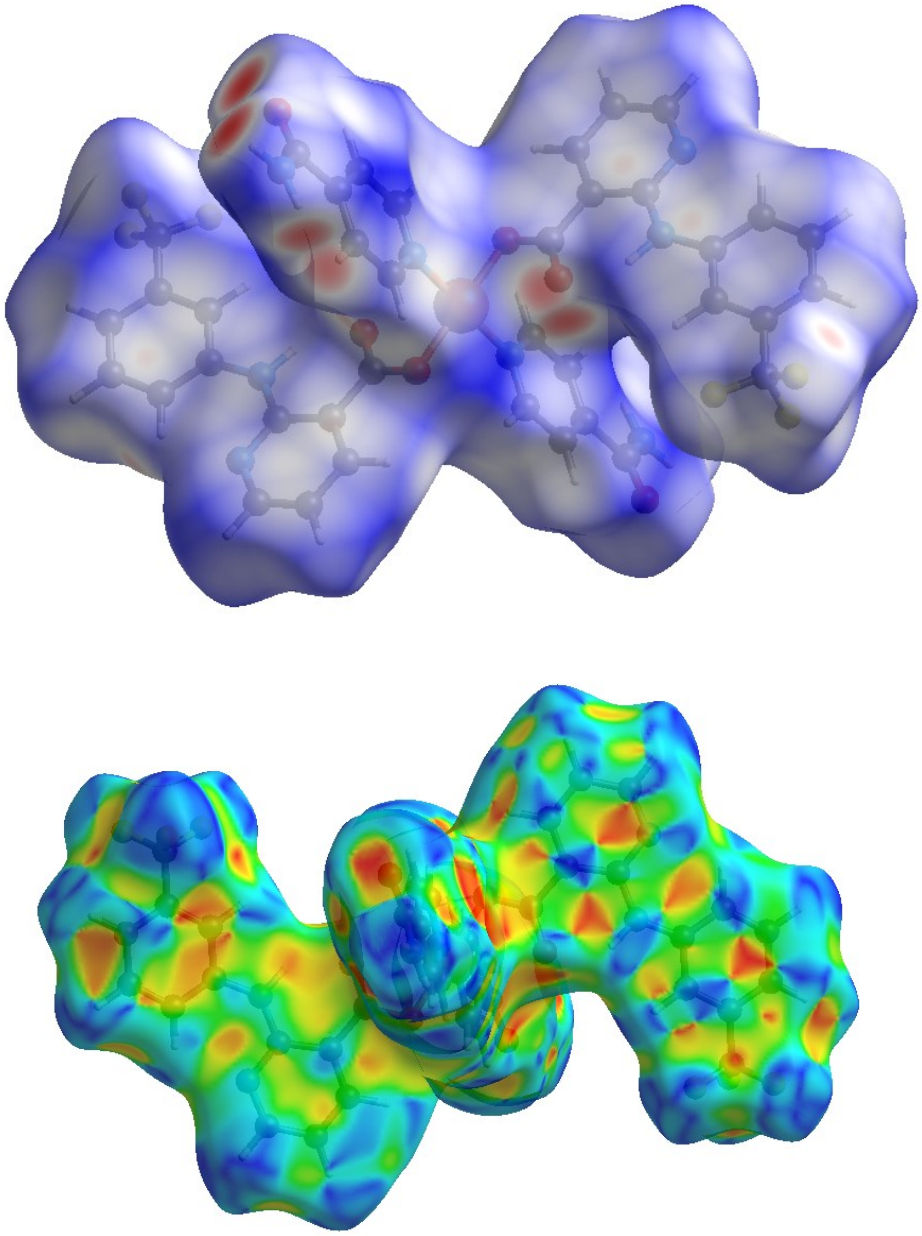


Figure S7. View of the three-dimensional Hirshfeld surface of **5** plotted over d_{norm} in the range -0.5661 to 1.1584 a.u. (top) and shape-index (bottom).

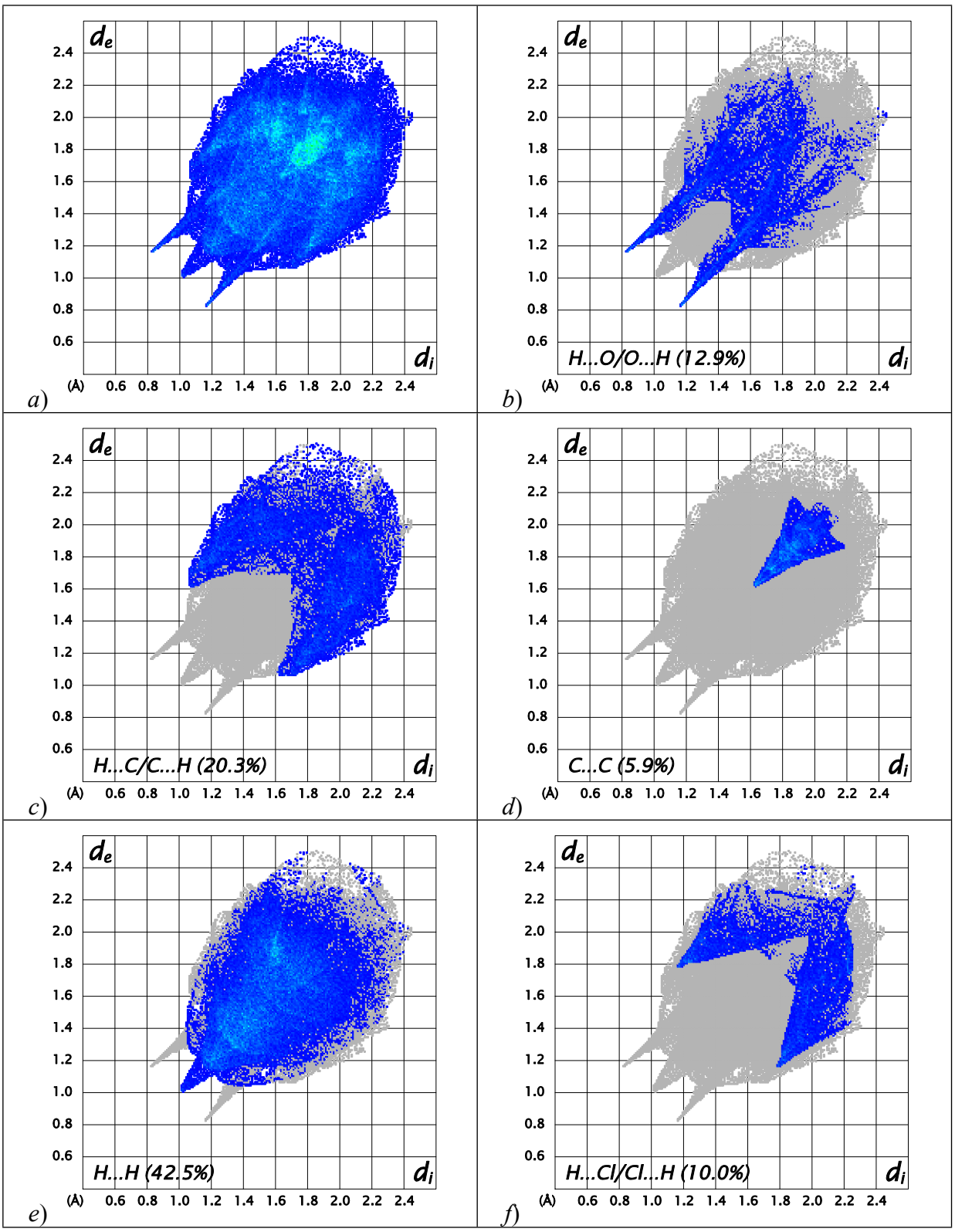


Figure S8. The full two-dimensional fingerprint plots of **1**, showing (*a*) all interactions, (b) H···O/O···H, (c) H···C/C···H, (*d*) C···C, (*e*) H···H, and (*f*) H···Cl/Cl···H interactions. The d_i and d_e values are the closest internal and external distances from given on the Hirshfeld surface contacts.

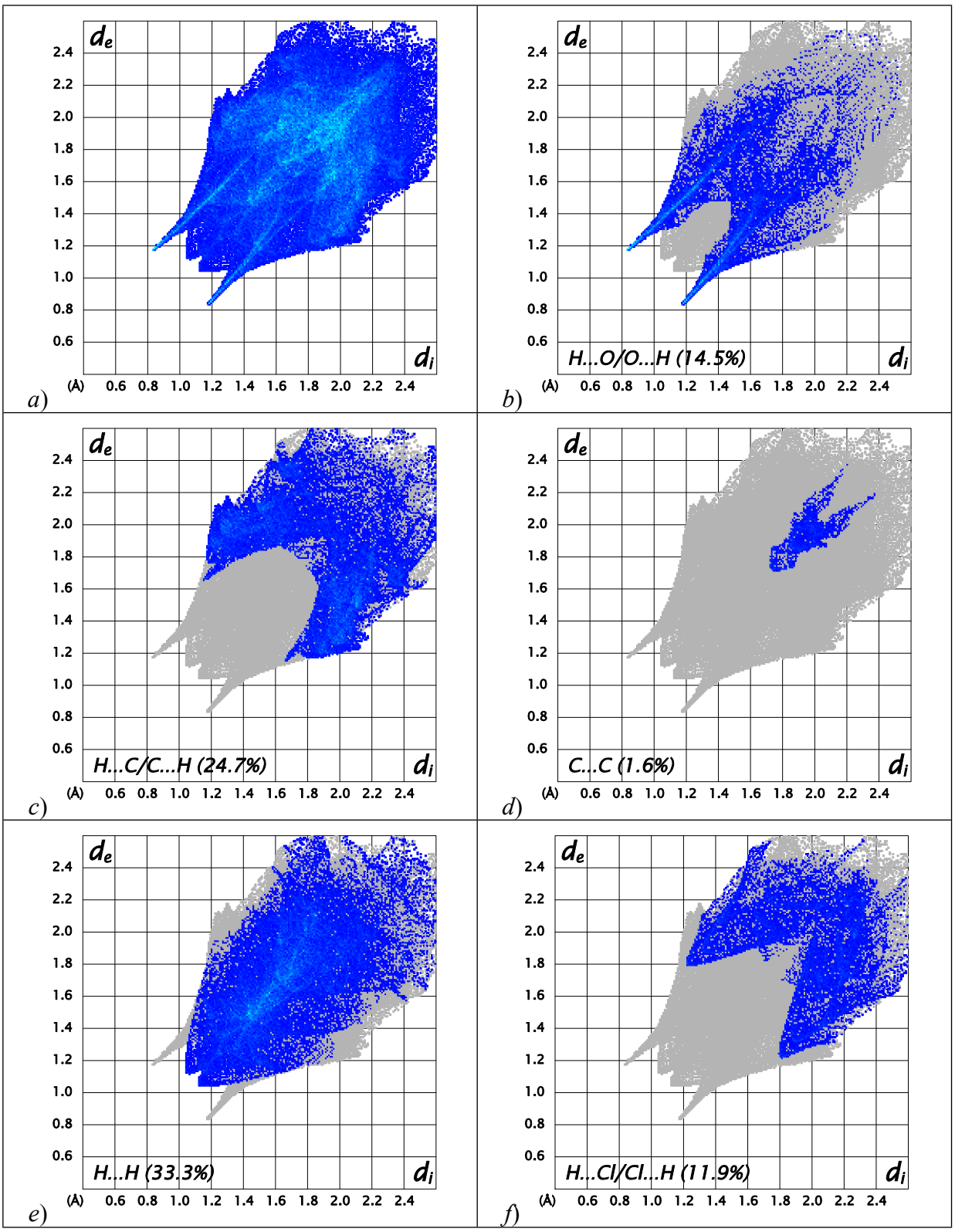


Figure S9. The full two-dimensional fingerprint plots of **2**, showing (*a*) all interactions, (b) H···O/O···H, (c) H···C/C···H, (*d*) C···C, (*e*) H···H, and (*f*) H···Cl/Cl···H interactions. The d_i and d_e values are the closest internal and external distances from given on the Hirshfeld surface contacts.

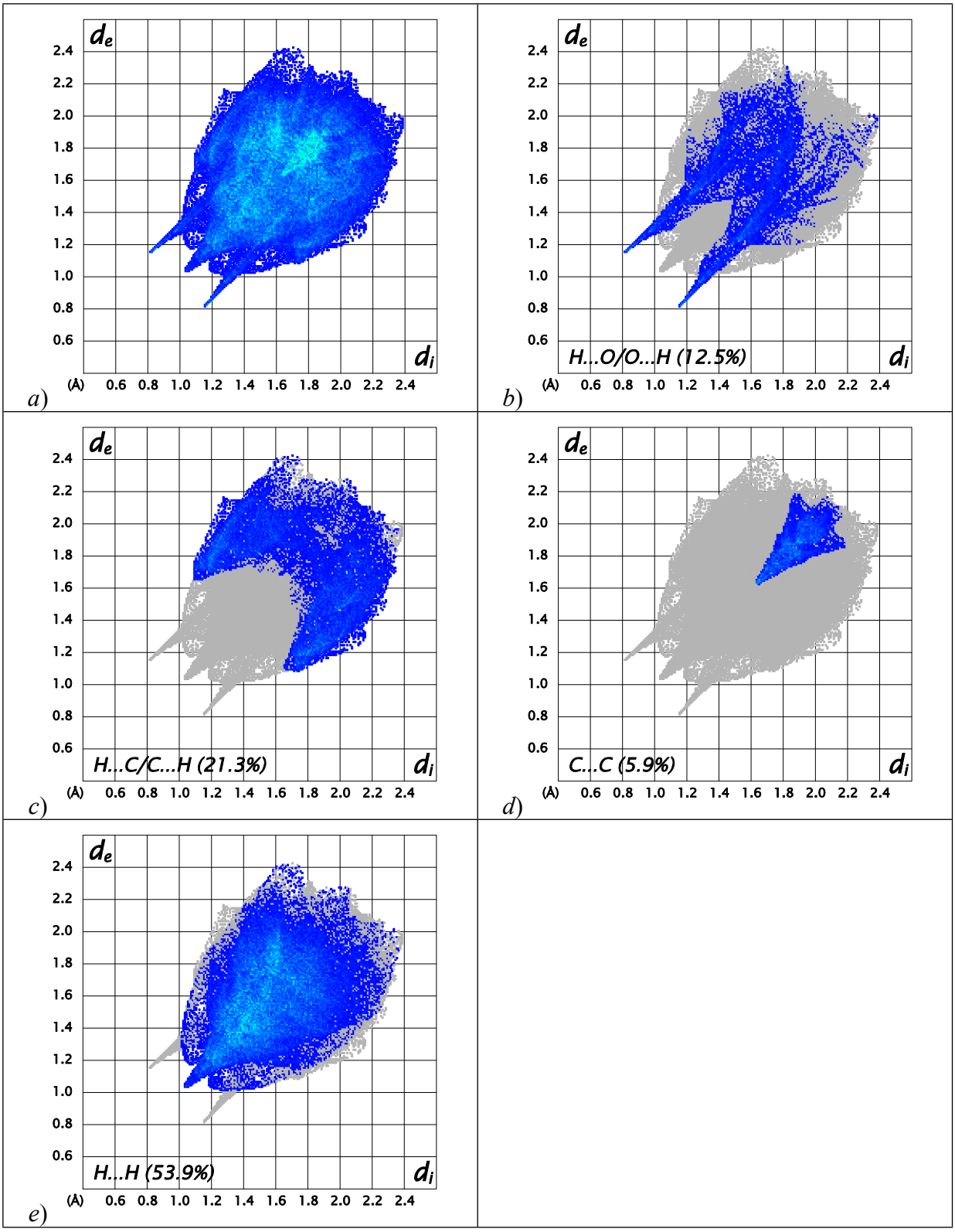


Figure S10. The full two-dimensional fingerprint plots of **3**, showing (*a*) all interactions, (b) H···O/O···H, (c) H···C/C···H, (*d*) C···C, and (*e*) H···H interactions. The d_i and d_e values are the closest internal and external distances from given on the Hirshfeld surface contacts.

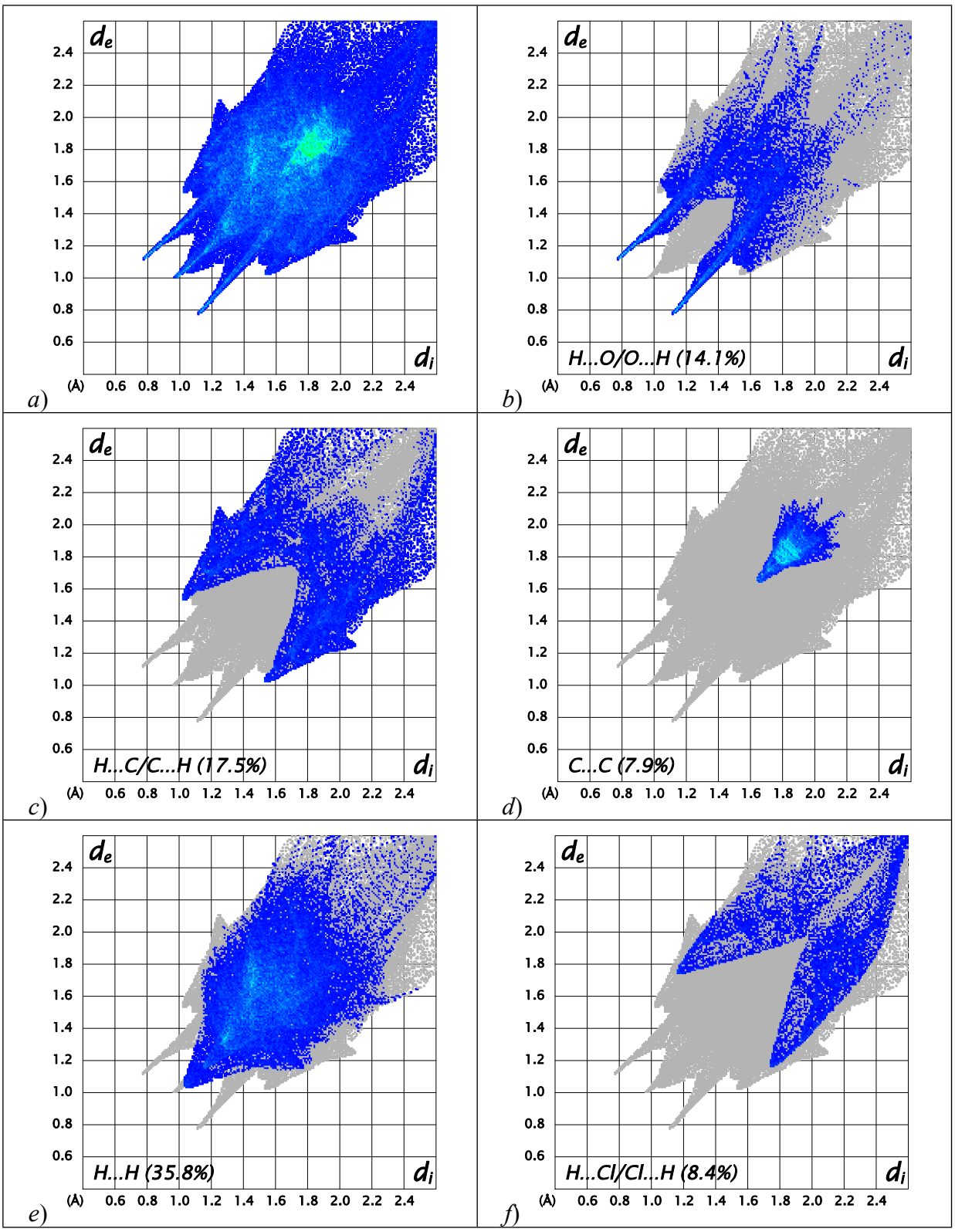


Figure S11. The full two-dimensional fingerprint plots of **4**, showing (*a*) all interactions, (b) H···O/O···H, (c) H···C/C···H, (*d*) C···C, (*e*) H···H, and (*f*) H···Cl/Cl···H interactions. The d_i and d_e values are the closest internal and external distances from given on the Hirshfeld surface contacts.

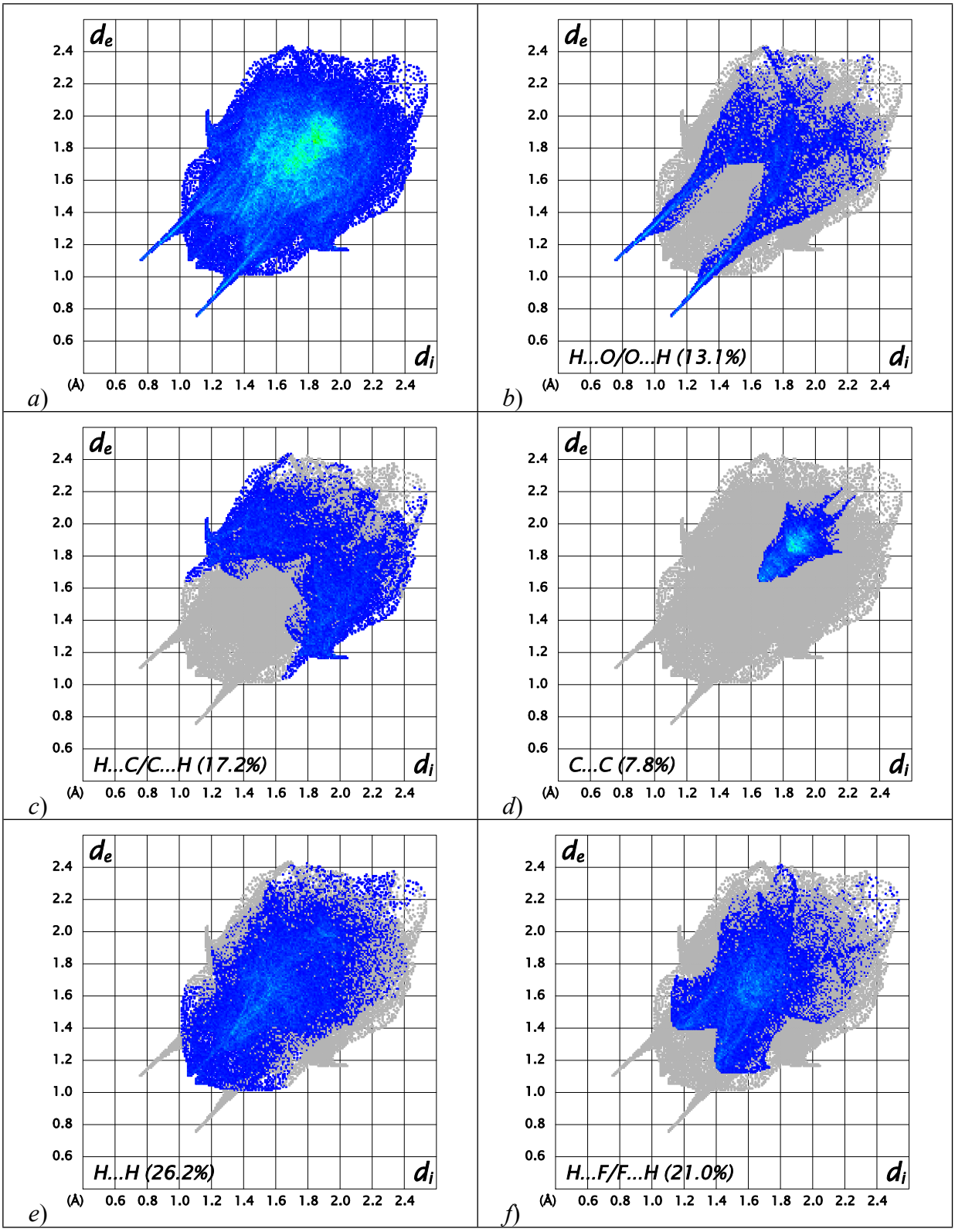


Figure S12. The full two-dimensional fingerprint plots of **5**, showing (*a*) all interactions, (b) H···O/O···H, (c) H···C/C···H, (*d*) C···C, (*e*) H···H, and (*f*) H···F/F···H interactions. The d_i and d_e values are the closest internal and external distances from given on the Hirshfeld surface contacts.

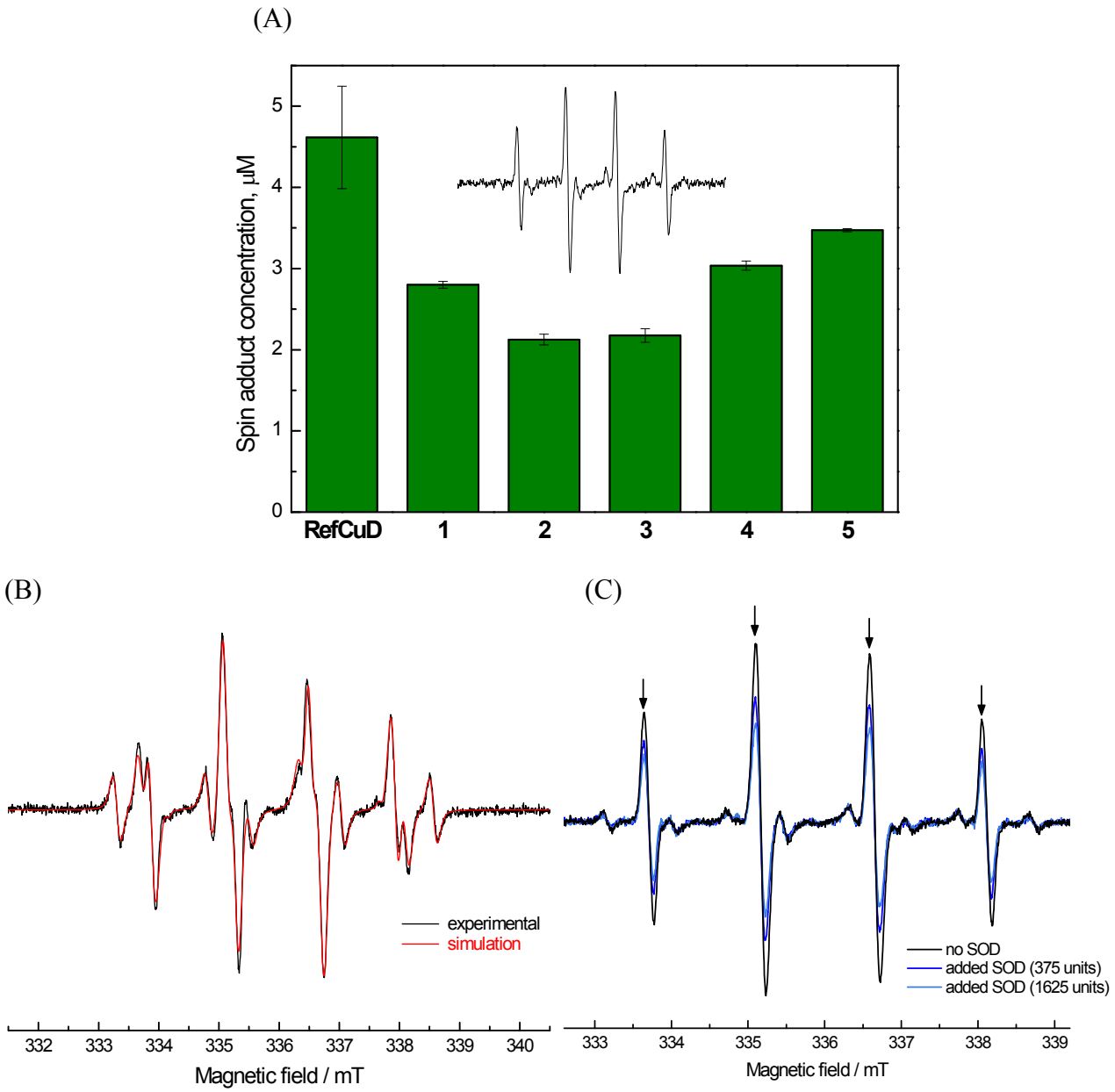


Figure S13. (A) The total concentration of DMPO spin-adducts observed in DMSO/water (1:4; v:v) solution of $CuCl_2$ (reference) or studied copper complexes containing DMPO spin trapping agent after the addition of hydrogen peroxide. Inset represents the EPR spectrum measured for the system 5 /DMPO/H_2O_2/H_2O/DMSO/air.(B) Experimental (–) and simulated (–) EPR spectra obtained in the system $CuCl_2$/H_2O_2/BMPO/DMSO:H_2O(1:4; v:v) under air. The simulation represents a linear combination of the EPR signals assigned to the following spin adducts: •BMPO-OH(1) (a_N=1.41 mT,a_H^{β}=1.26 mT, a_H^{γ}=0.06mT; g=2.0057; relative concentration60%); •BMPO-OH(2) (a_N=1.42 mT, a_H^{β}=1.56 mT, a_H^{γ}=0.05 mT; g=2.0057; 30%); •BMPO-CH_3 (a_N=1.52 mT, a_H^{β}=2.21 mT; g=2.0056; 10%). (C)Decline of the EPR spectra obtained in the system $CuCl_2$/H_2O_2/DMPO/DMSO:H_2O(1:4; v:v) under airupon the addition of SOD. Initial concentrations: c($CuCl_2$) = c(1-5) = 0.2 mM; c(DMPO/BMPO) = 0.02 M; c(H_2O_2) = 0.01 M.

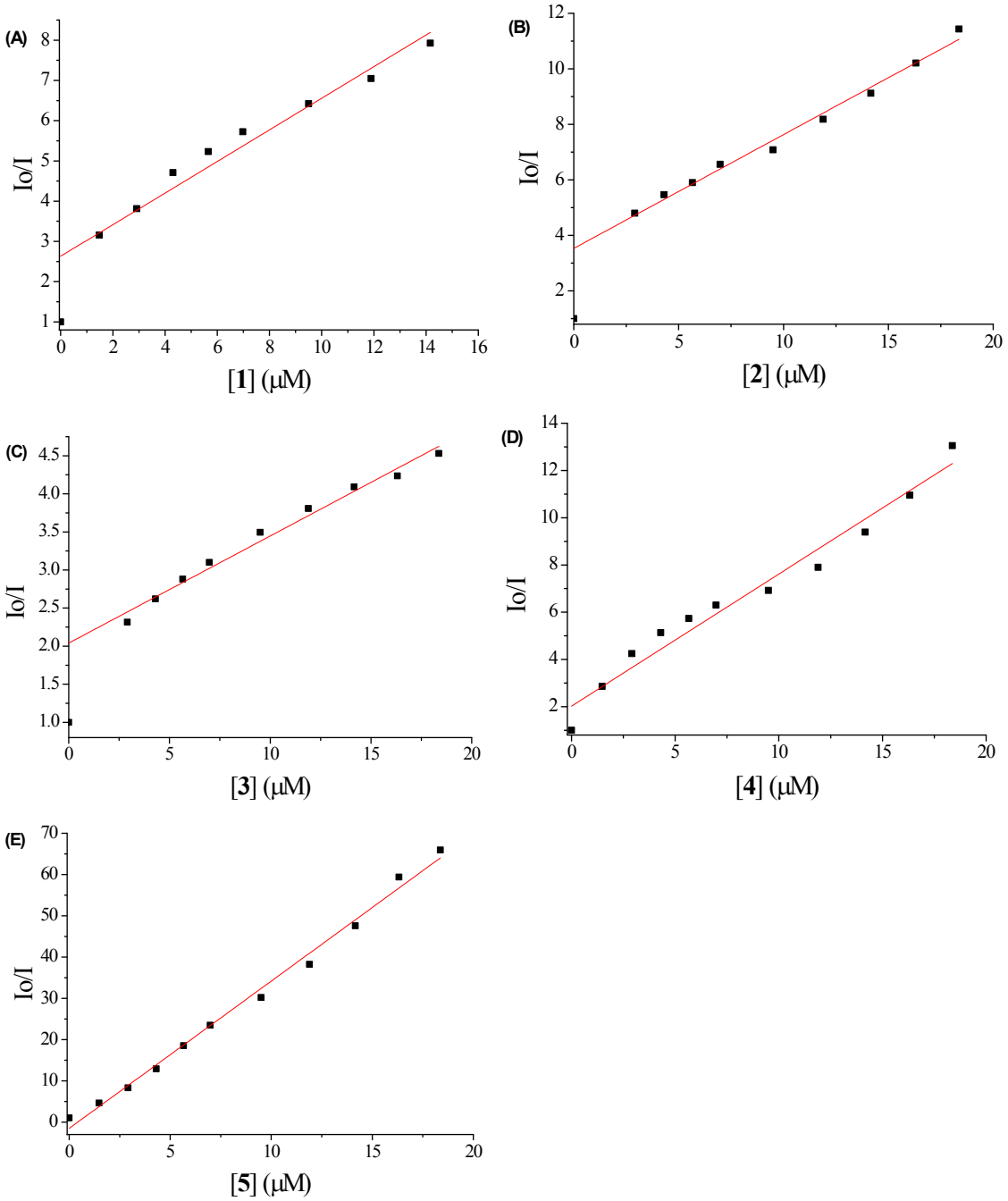


Figure S14. Stern-Volmer quenching plot of BSA for complex (A) **1**, (B) **2**, (C) **3**, (D) **4** and (E) **5**.

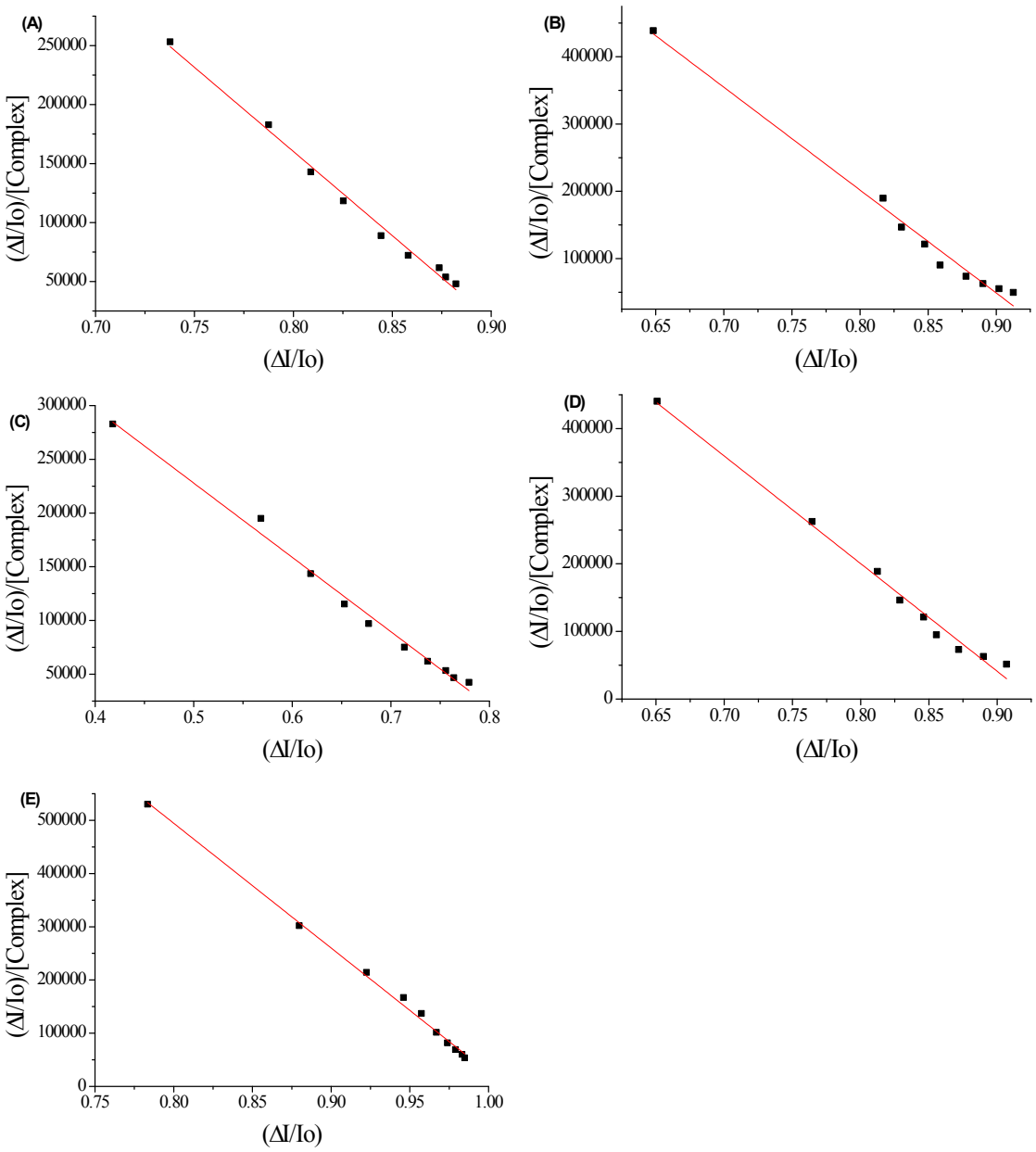


Figure S15. Scatchard plot of BSA for complex (A) **1**, (B) **2**, (C) **3**, (D) **4** and (E) **5**.

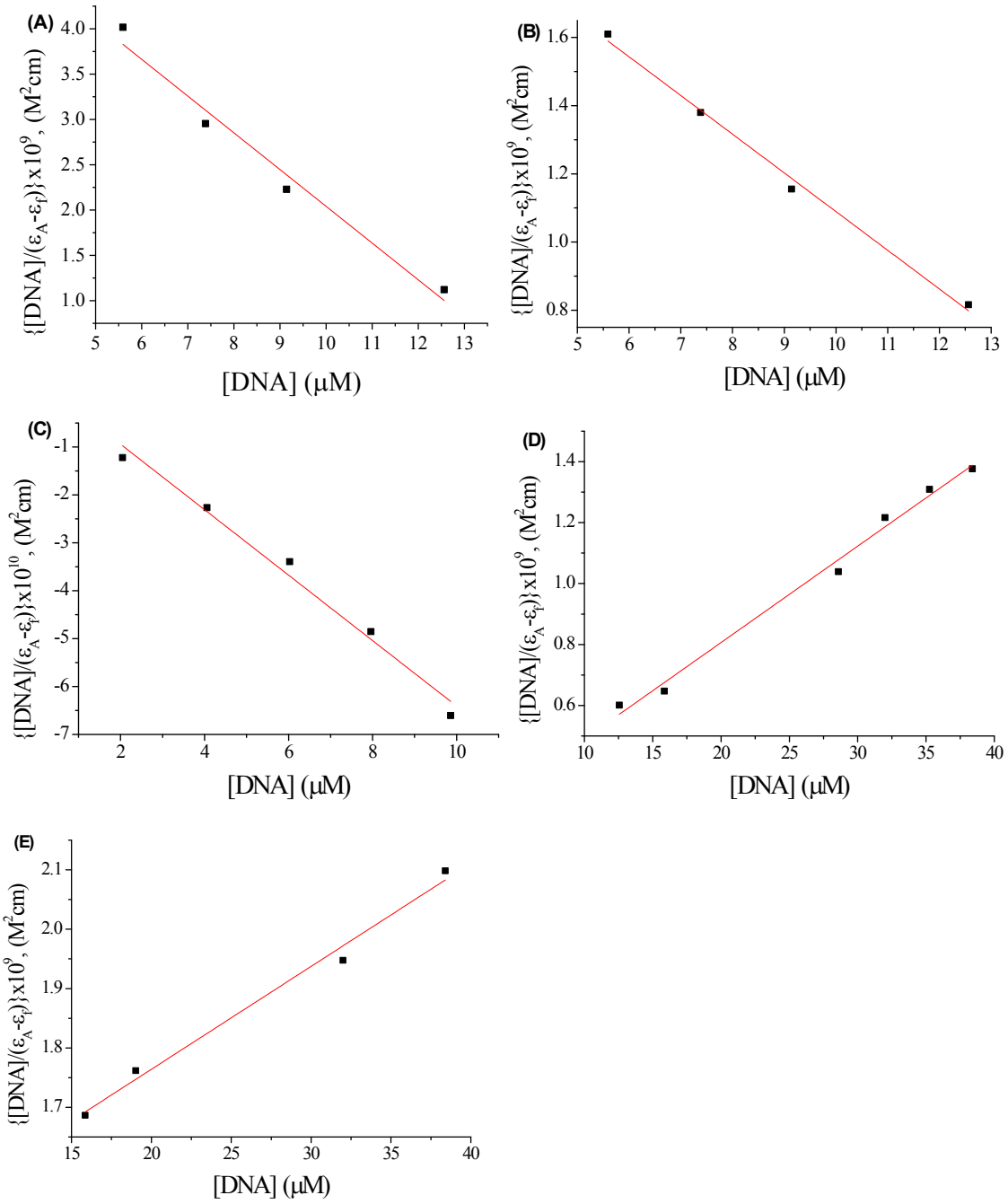


Figure S16. Plot of $[DNA]/(\varepsilon_A-\varepsilon_f)$ *versus* [DNA] for complex (A) **1**, (B) **2**, (C) **3**, (D) **4** and (E) **5**.

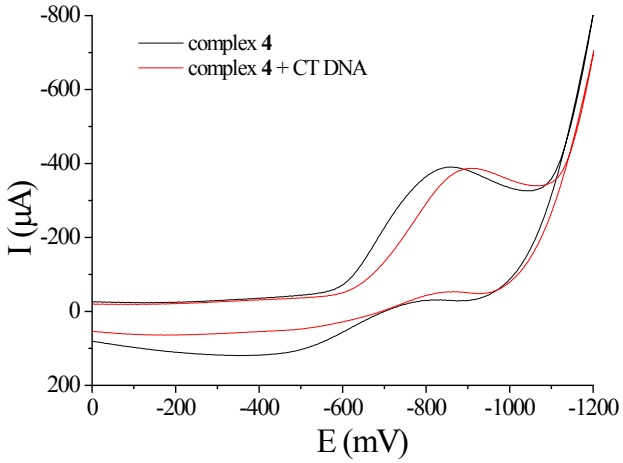


Figure S17. Cyclic voltammogram of 0.4 mM 1/2 DMSO/buffer (containing 150 mM NaCl and 15 mM trisodium citrate at pH = 7.0) solution of complex **4** in the absence (black line) or presence (red line) of CT DNA. Scan rate = 100 mV s^{-1}. Supporting electrolyte = buffer solution. The arrows show the changes upon addition of CT DNA.

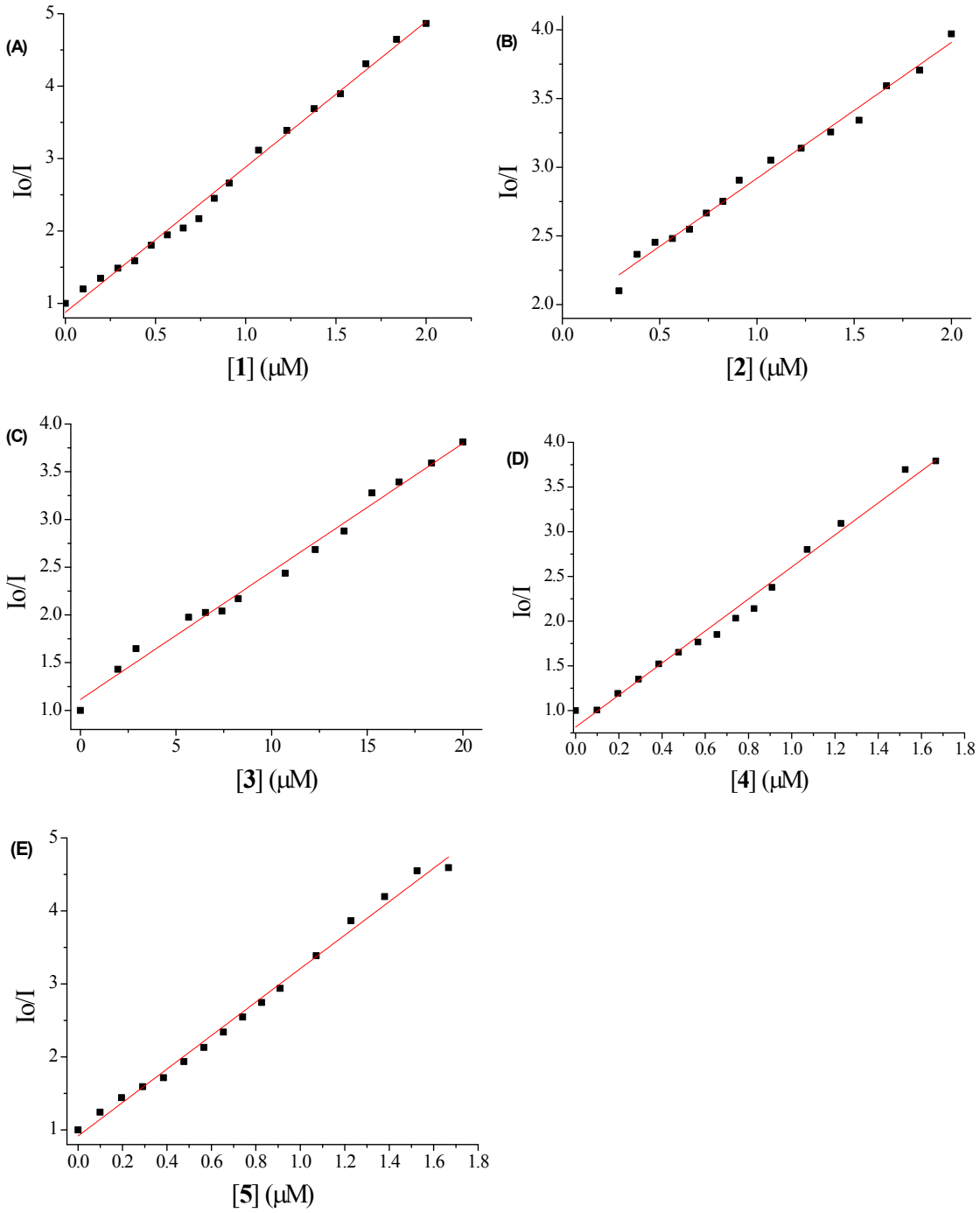


Figure S18. Stern-Volmer quenching plot of EB-DNA fluorescence for complex (A) **1**, (B) **2**, (C) **3**, (D) **4** and (E) **5**.