

DFT studies of substituted phenols cytotoxicity I. Para-substituted phenols

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Abstract

New type of cytotoxicity descriptors based on Cu(II) coordination ability of p-substituted phenols and subsequent O - Cu electron density transfer is introduced. Geometries of phenols and of their Cu(II) complexes are optimized at the DFT level of theory. The amount of electron density transfer is evaluated using the copper charge or the electron density Laplacian at the Cu-O bond critical point. The Cu-phenol interaction energy is a measure of the phenol reactivity. Statistical parameters for the linear dependence of the published cytotoxicity data on the above predictors are much better than the classical ones.

DFT studies of substituted phenols cytotoxicity I. *Para* -substituted phenols

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Keywords: copper; quantum chemistry; electron density transfer; cytotoxicity; QSAR

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1 Introduction

Phenolic compounds and their derivatives are constituents of numerous synthetic and natural chemicals such as drugs, cosmetics, dyes, agricultural products, pesticides, food additives etc. Their properties have been frequently studied at the molecular and cellular level. Phenolic-based compounds provide an excellent opportunity to examine their biological activities and to analyze corresponding mechanistic details in a comparative fashion using the quantitative structure activity relationship (QSAR) paradigm. It has been

useful in elucidating the mechanisms of chemical–biological interactions in various biomolecules, particularly enzymes, membranes, organelles and cells.^[1]

Selassie *et al.*^[2] studied the cytotoxicity of a series of 69 simple phenols in a fast growing murine leukemia L1210 cell line. They developed a Quantitative Structure–Activity Relationship (QSAR) model where the cytotoxicity of the substituted phenol was evaluated as $\log(1/IC_{50})$, with IC_{50} being its concentration that inhibited cell growth by 50%. These values were correlated with Brown-Hammett parameters (σ^+), the HOMO–LUMO energy difference ($L-H$ gap), the logarithmic n-octanol - water partition coefficients ($\log P$) and homolytic bond dissociation energies (BDE). Phenols with electron withdrawing substituents were excluded from the analyses. BDE values were calculated at B3LYP/6-31G**//AM1 level of theory with effective core potentials used for bromine- and iodine-substituted molecules. $L-H$ gap values were obtained by the semiempirical AM1 method only. Despite these restrictions, the developed QSAR models granted surprisingly good results with 17 – 27 outliers depending on the descriptors used. It seems that the above mentioned cytotoxicity descriptors cannot produce better results.

Alagona and Ghio^[3] evaluated the antioxidant activity of various sites of prenylated pterocarpan according to their Cu^{2+} coordination ability using the metal ion affinity (MIA) values determined by B3LYP calculations. Spin density of the cation upon ligand coordination became negligibly low, whereas the ligand spin density approached 1. Thus, the ligand was oxidized to a radical cation (Ligand^{*+}), while $Cu(II)$ was reduced to $Cu(I)$. In agreement with experimental investigations, the higher antioxidant activity of individual compounds and their reaction sites can be assigned to higher MIA values and higher reducing character towards $Cu(II)$. The Cu^{2+} ion serves as a probe to estimate the extent of the electron density transfer only and the optimized structures need not correspond to any real complex. Mammino^[4] analogously tested antioxidant ability of various sites of hyperjovino A. Another modification of the above-mentioned method by including Laplacians at $Cu-N$ bond critical points (BCP) as the measure of the corresponding electron density transfer has been used for both N centres of a series of *para*-phenylene diamine antioxidants.^[5]

Other 1st row transition metal ions were also used in the role of similar probes for the compounds with oxygen active sites. B3LYP, M06-2X, BHandHLYP, MPWLYP1M and G96LYP methods were used to study antioxidant properties of kanakugiol through its $Cu(II)$ and $Co(II)$ coordination ability^[6] as well as lucidone, linderone and methylinderone through their $Fe(II)$ coordination ability.^[7] Subsequently the authors continued in studying antioxidant properties of butein and homobutein^[8], chalcone derivatives kanakugiol and pedicellin^[9] by considering their $Fe(II)$ and $Fe(III)$ coordination ability using the B3LYP method. A systematic B3LYP study of a series of *para*-phenylene diamine antioxidants with the late 1st row transition metals in various spin states^[10] has shown that using these $M(II)$ probes yield very similar results of antioxidant effectiveness.

This method was used for the relative cytotoxicity estimation of various sites of model rutile nanoparticles as well.^[11] It was shown that the experimentally observed higher cytotoxicity of rod-like nanoparticles in comparison with the spherical ones might be explained by the higher electron density transfer to the interacting cells (as indicated by the extent of the electron density transfer to a Cu^{2+} probe), whereas MIA reflects the reactivity of individual active sites. The aim of our recent study is the application of this method to predict the cytotoxicity of *p*-substituted phenols. Our results will be compared with standard QSAR correlations used by Selassie *et al.*^[2]

2 Method

Geometries of 24 neutral *p*-substituted phenol (Ph) molecules (see Table 1) in singlet ground states, as well as of their the²[Ph... Cu]²⁺ complexes in doublet ground states (the left superscript denotes their spin multiplicity) with Cu bonded exclusively to O atoms, were optimized using B3LYP hybrid functional^[12] with the DGDZVP pseudopotential and basis set for iodine^[13] and standard 6-311G* basis sets from the Gaussian library^[14] for the remaining atoms. Stability of the obtained geometries was confirmed by vibrational analysis (no imaginary vibrations). Charges and spin populations of copper atoms were evaluated using Mulliken (MPA)^[15] and Natural Bond Orbital (NBO)^[16] population analyses. Gaussian09 software was used for all

quantum-chemical calculations.^[14]

Table 1. Experimental toxicity data of Selassie *et al.* ^[2] for 4-R-phenols.

Data set	R	log(1/IC ₅₀)
A	-H	3.27
	-CH ₃	3.85
	-C ₂ H ₅	3.86
	-C ₃ H ₇	4.04
	-C ₄ H ₉	4.33
	-C ₅ H ₁₁	4.47
	-C ₇ H ₁₅	4.49
	-C ₈ H ₁₇	4.62
	-C ₉ H ₁₉	4.75
	-C(CH ₃) ₃	4.09
	-OC ₆ H ₅	4.97
B	-I	3.86
	-OCH ₃	4.48
	-OC ₂ H ₅	4.64
	-OC ₃ H ₇	4.85
	-OC ₄ H ₉	5.20
	-OC ₆ H ₁₃	5.50
	-NO ₂	3.45
	-F	3.83
	-NH ₂	5.09
	-OH	4.59
	-Cl	4.29
C	-Br	4.20
	-CN	3.44
	-CONH ₂	2.48
	-SO ₂ NH ₂	2.50
	-CHO	3.08
	-NHCOCH ₃	3.73

Alternatively, the charge and spin population of Cu was evaluated in terms of Quantum Theory of Atoms-in-Molecules (QTAIM)^[17] (see Supplementary Information for details). The O - Cu electron density transfer was alternatively estimated as the Laplacian of the electron density ($[\rho]^2$) at Cu - O bond critical points (BCP) using AIMAll software.^[18] The MOLDRAW software^[19] was used for geometry manipulation and visualisation purposes.

MIA is evaluated as the interaction energy

$$E_{\text{int}} = E_{\text{complex}} - E_{\text{Cu(II)}} - E_{\text{Ph}} \quad (1)$$

where E_{complex} is the DFT energy of the $[\text{Ph} \dots \text{Cu}]^{2+}$ complex, $E_{\text{Cu(II)}}$ is the DFT energy of the Cu^{2+} ion and E_{Ph} is the DFT energy of the p-substituted phenol molecule.

The standard linear regression analysis was used to obtain the regression parameters a and b of the relation

$$\log(1/IC_{50}) = aX + b \quad (2)$$

where $\log(1/IC_{50})$ are the experimental toxicity data of Selassie *et al.* ^[2] and X stands for predictor variables under study. The goodness of fit is evaluated by standard statistical parameters such as the coefficient of determination R^2 or the critical value α of the F-test.

3 Results and discussion

Our study deals with 28 p-substituted phenols of the set of Selassie *et al.* [2] despite five to seven of them with electron withdrawing substituents (-NO₂, -NH₂, -CN, -Cl, -Br, -CONH₂, -CHO, -NHCOCH₃, -SO₂NH₂) were omitted from their analyses. We suppose that the phenols cannot be investigated as a whole. Therefore we have classified the whole group into three datasets as follows (Table 1):

1. Dataset A contains electron donating substituents such as R = H or alkyl. The iodine atom can be assigned to this dataset due to its low electronegativity as well. Surprisingly, also the phenoxy substituent behaves as the remaining ones of this dataset probably due to neutralization of opposite electron withdrawing properties by its bonded aromatic ring (resonance effect). Therefore this dataset contains 12 molecules.
2. Dataset B contains electron withdrawing substituents such as R = alkoxy and hydroxyl, halogens (except I), cyano-, nitro- and amino- groups, altogether 12 molecules (some of them were omitted by Selassie *et al.* [2]).
3. Dataset C contains four molecules (omitted by Selassie *et al.* [2]) with substituents containing C=O or S=O bonds which might affect the toxicity by different mechanisms than phenols. This dataset is too small to be treated by regression analyses.

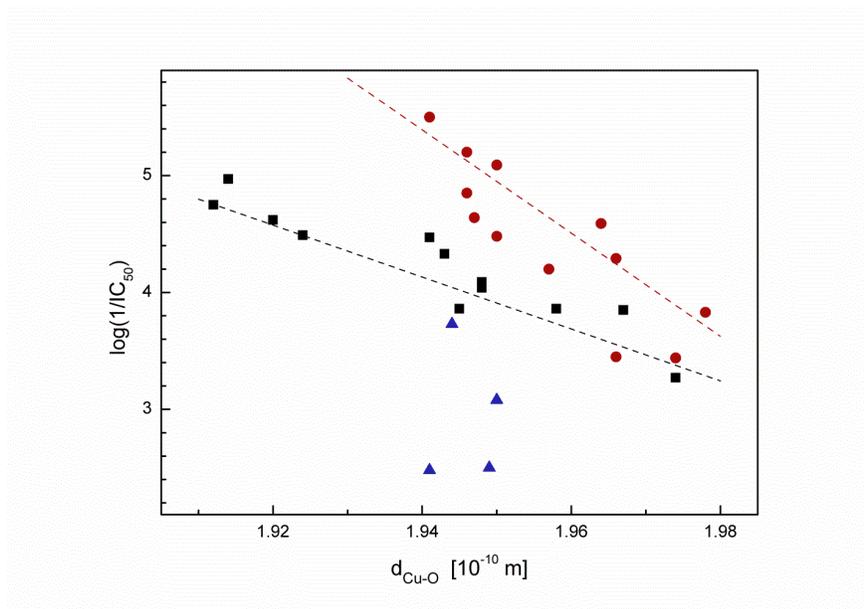


Figure 1. Plot of the relation between p-phenols toxicity, $\log(1/IC_{50})$, and Cu-O bond lengths, d_{Cu-O} , of data sets A (black squares), B (red circles) and C (blue triangles). Dashed lines correspond to linear parameters in Table 2.

Table 2. Regression parameters of Eq. (2) with standard deviations, coefficients of determination R^2 , root-mean-squares (RMS) errors and critical values α of the F-test for various predictor variables X as well as A and B datasets (see Table 1).

X	Data set	a ^a	b	R ²	RMS error	α
d_{Cu-O} [10^{-10} m]	A	-22.2±2.6	47.2±5.1	0.86653	0.030	6.8×10^{-6}
	B	-46.2±8.8	95. ±17.	0.70541	0.13	3.8×10^{-4}
Q_{MPA}	A	-20.3±2.3	23.7±2.2	0.87635	0.028	4.6×10^{-6}
	B	-40.7±6.9	44.0±6.7	0.75246	0.11	1.6×10^{-4}

X	Data set	a ^a	b	R ²	RMS error	α
Q_{NBO}	A	-24.2±2.4	27.6±2.3	0.90003	0.023	1.6×10 ⁻⁴
	B	-38.1±7.4	41.8±7.3	0.69754	0.13	4.6×10 ⁻⁴
Q_{QTAIM}	A	-23.1±2.2	25.2±2.0	0.91125	0.020	8.7×10 ⁻⁷
	B	-43.2±5.0	44.2±4.6	0.86900	0.057	6.2×10 ⁻⁷
[?] ² ρ_{BCP} [e/bohr ⁵]	A	14.3±1.7	-3.24±0.88	0.86661	0.030	6.8×10 ⁻⁶
	B	29.6±6.0	-10.2±3.0	0.68103	0.14	5.8×10 ⁻⁴
E_{int} [kJ/mol]	A	(-8.86±0.70) ×10 ⁻³	-4.36±0.68	0.93598	0.015	1.7×10 ⁻⁷
	B	(-6.85±0.81) ×10 ⁻³	-2.05±0.77	0.86562	0.058	7.1×10 ⁻⁶
σ^+	A	-1.98±0.68	3.73±0.20	0.40400	0.14	1.5×10 ⁻²
	B	-0.85±0.15	4.14±0.11	0.74386	0.11	8.8×10 ⁻⁴
log P	A	0.258±0.064	3.29±0.25	0.58097	0.095	2.4×10 ⁻³
	B	0.16±0.19	4.15±0.41	0.02374	0.45	4.1×10 ⁻¹
$L-H$ gap [eV]	A	2.11±0.87	23.9±8.1	0.30805	0.16	3.6×10 ⁻²
	B	2.12±0.60	23.7±5.5	0.50888	0.21	5.5×10 ⁻³
BDE [kcal/mol]	A	-0.359±0.082	3.51±0.18	0.61973	0.086	1.4×10 ⁻³
	B	-0.138±0.023	3.97±0.13	0.75527	0.10	1.5×10 ⁻⁴

Remarks

^aIn units reciprocal to X units

Molecular graphs of the the²[Ph... Cu]²⁺ complexes (see Fig. S1 of Supplementary information) suggest the Cu-O distance, $d_{\text{Cu-O}}$, as the most simple predictor variable (Fig. 1, Table S1 of Supplementary information) which might reflect the strength of the phenolic oxygen bonding with the interacting cell. The corresponding statistical parameters of Eq. (2) are fairly good, especially for the dataset A (Table 2).

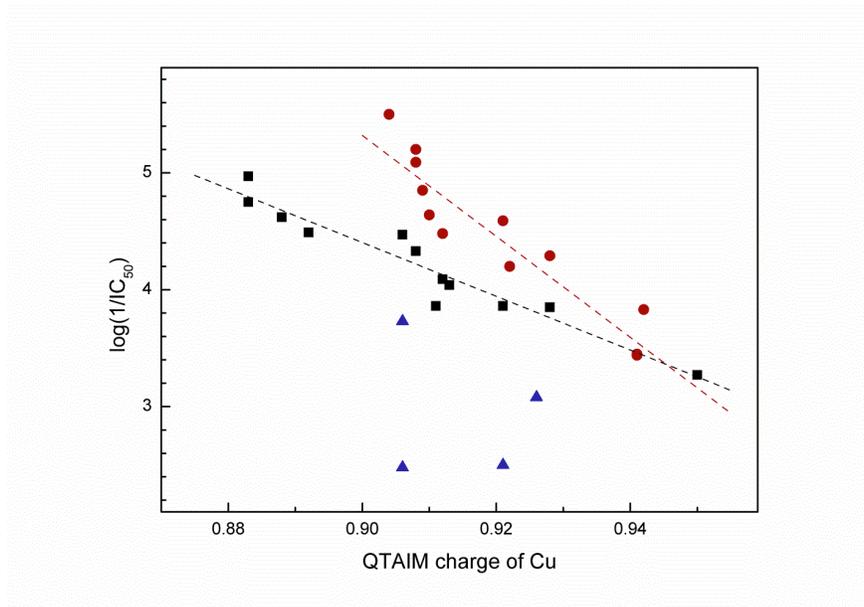


Figure 2. Plot of the relation between p-phenols toxicity, $\log(1/IC_{50})$, and QTAIM charges of Cu atoms of data sets A (black squares), B (red circles) and C (blue triangles). Dashed lines correspond to linear parameters in Table 2.

Copper charges in the $[\text{Ph} \dots \text{Cu}]^{2+}$ complexes (as compared with original Cu^{2+} ion charges) are a measure of the O - Cu electron density transfer (Table S1 in Supplementary information) and reflect the phenol toxicity (Table 2, Figs. 2, S2 and S3 in Supplementary information). The QTAIM charges exhibit better statistical parameters for Eq. (2) than the NBO and MPA ones, especially for the dataset A (Table 2). Nevertheless, in all cases their fit is better than for the Cu-O distances above.

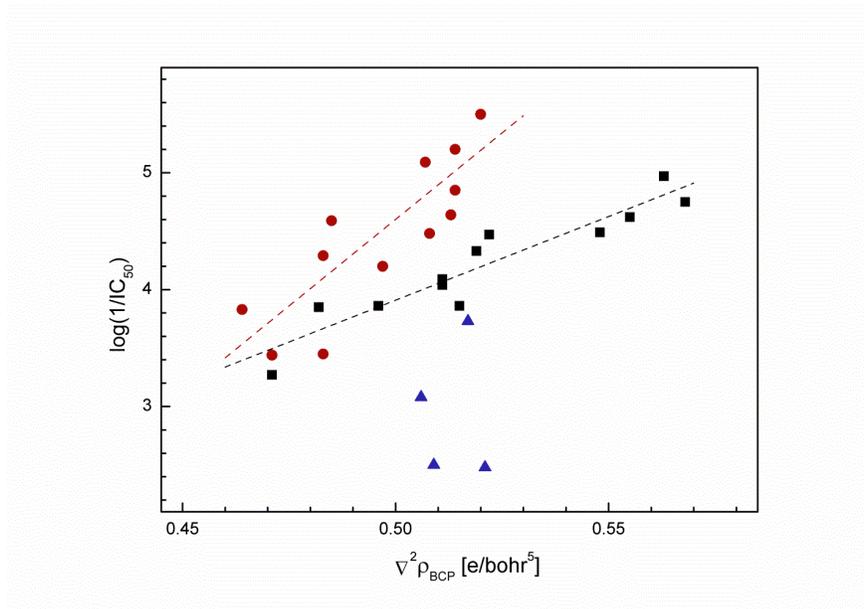


Figure 3. Plot of the relation between p-phenols toxicity, $\log(1/IC_{50})$, and BCP electron density Laplacians of Cu-O bonds, $[\nabla^2] \rho_{\text{BCP}}$, of data sets A (black squares), B (red circles) and C (blue triangles). Dashed lines correspond to linear parameters in Table 2.

Alternatively, the extent of the O - Cu electron density transfer can be evaluated in terms of the BCP electron density Laplacian, $[\nabla^2] \rho_{\text{BCP}}$, of the Cu - O bond (Fig. 3, Tables 2 and S1 in Supplementary information). Their correlation with the cytotoxicity according to Eq. (2) exhibits a little bit worse statistical characteristics than in the case of QTAIM charges for both A and B datasets.

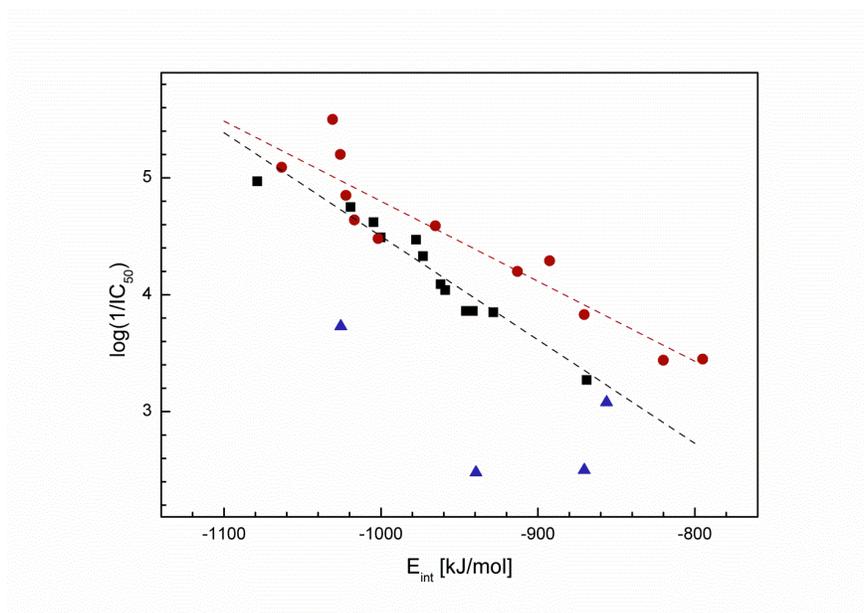


Figure 4. Plot of the relation between p-phenols toxicity, $\log(1/IC_{50})$, and interaction energies, E_{int} , of data sets A (black squares), B (red circles) and C (blue triangles). Dashed lines correspond to linear parameters in Table 2.

Finally, the cytotoxicity correlation with the interaction energy data, E_{int} , (Table S1 in Supplementary information) as a measure of the phenol reactivity^[11] according to Eq. (2) exhibit the best statistical parameters (Table 2, Fig. 4) for the dataset A.

There are significant differences between the regression parameters of the Eq. (2) for both A and B datasets. Except for E_{int} , the B dataset ones are nearly doubled. In agreement with worse statistical parameters for the B dataset, a similar relation holds for their standard deviations as well. With few exceptions, the B dataset cytotoxicity values are higher than the A ones for similar predictor values whereas the C ones are even lower. The rightness of inclusion of the iodine substituent into the A dataset is confirmed by comparing the corresponding data evaluated by the Eq. (2) by using its regression parameters both for A and B datasets (Table S3 of Supplementary information).

We performed regression analysis for the predictor data of the Brown-Hammett parameters (σ^+), the HOMO-LUMO energy difference ($L-H$ gap), the logarithmic n-octanol - water partition coefficients ($\log P$) and homolytic bond dissociation energies (BDE) used by Selassie *et al.*^[2] as well. The obtained results for the regression parameters of Eq. (2) and related statistical data are presented in Table 2 and Figs. S4 – S7 of Supplementary information. In all cases these variables offer much worse statistical parameters than our predictors related to a Cu(II) probe. The distribution of A and B dataset data is more chaotic despite the b parameter of the Eq. (2) for both datasets is nearly identical.

Finally it can be concluded that the QSAR model based on quantum-chemical calculations of the Cu(II) coordination ability of p-substituted phenols offers much better results than classical treatments.^[2] The predictors based on the O - Cu electron density transfer, such as copper charges and BCP electron density Laplacian of the Cu-O bond in the $2[Ph...Cu]^{2+}$ complexes, as well as the corresponding interaction energy are able of a very good toxicity prediction. The p-substituted phenols with electron withdrawing and electron donating substituents must be treated separately due to different linear dependencies of both groups. Nevertheless, there may be more than one mechanism for various types of toxicity as indicated by the phenols of the C dataset. Further experimental as well as theoretical studies in this field are desirable.

Declarations of interest

None.

Funding information

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Appendix. Supplementary data

Supplementary data to this article can be found online at <https://>

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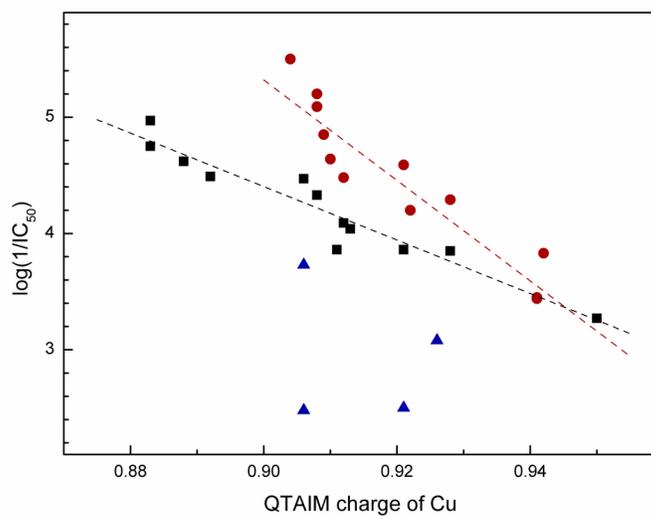
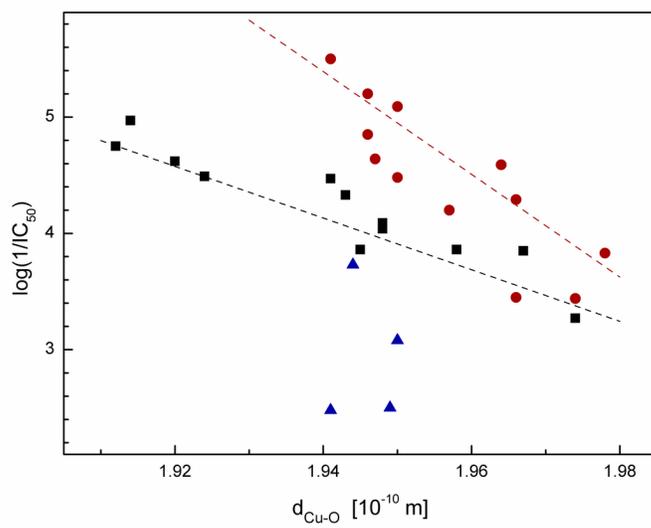
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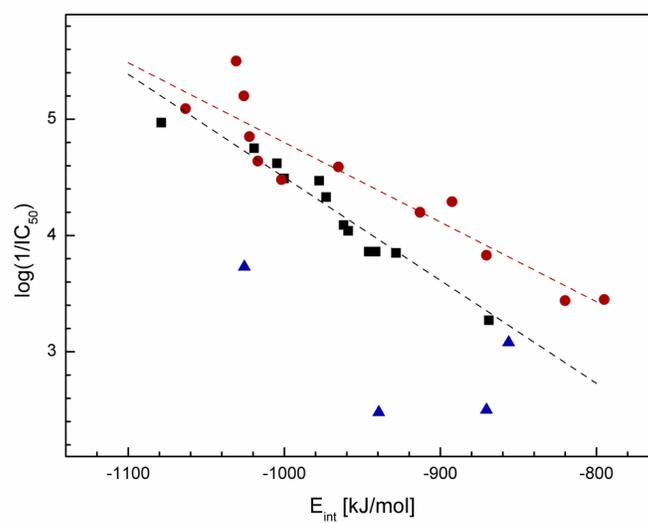
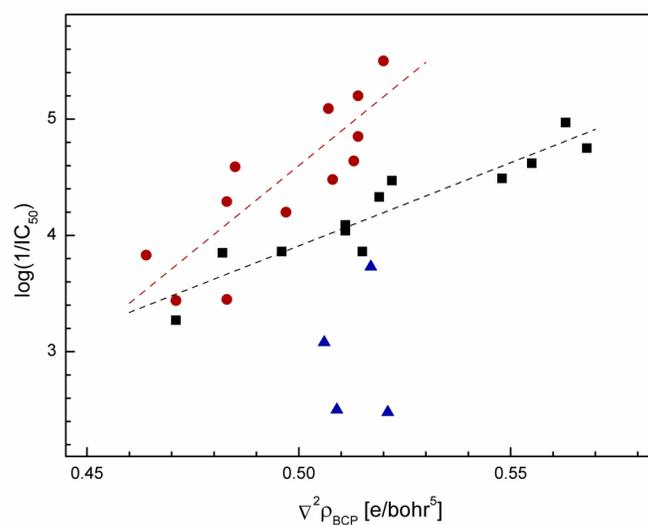
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