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Global Structure–Activity Analysis in Drug Development Illustrated for Active Cu/Zn Superoxide Dismutase Mimics

Cornelia G. Palivan,*[a] Vimalkumar Balasubramanian,[a] and Bernard A. Goodman[b]

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The analysis of the spectroscopic and electrochemical properties of all reported classes of Cu/Zn superoxide dismutase mimics (SODm) makes it possible to distinguish the geometric specificity of highly active compounds. A linear relationship with EPR parameters characterises the geometry of the first coordination sphere around the metal, which favours catalytic activity. Combined with the appropriate redox potential values, the model of geometric conformation of highly active Cu/Zn SODm serves to streamline further synthetic efforts. This global analysis represents a new approach to treat all classes of compounds intended for a specific biological activity in order to obtain general features that could orient the process of drug discovery.

Recent developments in theoretical chemistry allow the calculation of structures and physical properties of transition-metal complexes in advance of their synthesis, with the result that synthetic efforts can be devoted to the preparation of molecules that are most likely to possess the desired characteristics. However, this approach has not been widely used in novel drug development, because of the inability of theoretical methods to predict biological activity, and it has been largely devoted to refining existing products.[1] An alternative approach is based on structure–activity relationships (QSAR), which has been applied successfully to some particular classes of ligands. However, there is no general QSAR approach that analyses all classes of compounds proposed for a specific biological activity.[2]

Herein we present an essential step towards the optimisation of a drug development strategy: a global structure–activity analysis, which, contrary to QSAR, is applied to all classes of compounds already proposed for a specific biological activity. We discuss how statistical analysis can be applied to identify physical properties associated with biological activity for a family of drugs known as superoxide dismutase mimics (SODm) that act as effective scavengers of the superoxide free radical anion ($O_2^{\cdot-}$), one of the major reactive oxygen species (ROS) responsible for oxidative stress in aerobic organisms.[3]

Cu/Zn superoxide dismutase (SOD) is one of the metal protein families that efficiently decreases the steady-state concentration of $O_2^{\cdot-}$ by a very fast process (at nearly diffusion-limited rates), which is nearly independent of pH (over the range 5.0–9.5).[4] However, SOD can not be administrated directly, because of its unfavourable pharmacological profile.[5] One way to circumvent this shortcoming is to use low-molecular-mass complexes with SOD-like activity, as the metal site of the protein has been proved to be the active catalytic region.[6] The advantages of this approach have been recognised in terms of overcoming immunogenic problems associated with protein administration, tissue permeability and potential in vivo stability.[7] Therefore, intensive efforts have been made for many years to obtain compounds with high SOD activity. Copper-based SODm have been proposed, either as binuclear or mononuclear metal complexes, with several classes of ligands, including derivatives of imidazoles,[8] amines,[8g,8h,8n,9] pyridines,[8c,8g,8m,8v,8w,9a–9c,9e,9g,10] sulfonamides,[8a,11] peptides,[8q,8r,10e,12] imines,[9f,13] salicylate,[8j,14] cyclodextrin,[15] curcumin,[16] triazines,[17] quinoline[9h,10e,18] and others.[19] Despite these efforts to obtain better SODm, there are only a few studies that have tried to connect the biological activity with electrochemical[10a] or spectroscopic data,[17a,17b] and they are limited to particular classes of ligands.

The question we address now is "Is there a general relationship between geometrical parameters describing the environment of the metal for SODm, or are there a number of specific ligand arrangements that induce a high level of biological activity?" Our strategy for establishing a structural model for high SOD activity was to analyse (most of) the reported SODm statistically to determine whether spectroscopic parameters (which characterise the metal co-

[a] Department of Chemistry, University of Basel,
Klingelbergstrasse 80, 4056 Basel, Switzerland
Fax: +41-612673855
E-mail: cornelia.palivan@unibas.ch
[b] Department of Environmental Research, Austrian Research Center Seibersdorf,
2444 Seibersdorf, Austria

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ordination) and redox potentials are consistently related to each other or to the activity of a complex. We started with the hypothesis that the ligand dictates the catalytic activity by promoting or preventing a particular conformational structure. Consequently, we expect that any movement of one of the first coordination sphere nuclei out of the equatorial plane will change the ability of the corresponding complex to function as a catalyst.

This hypothesis is in agreement with the SOD redox catalytic cycle, which involves a reversible conformational change at the active site. The geometry of the first coordination sphere around the metal ion is imposed by the ligands in a way that facilitates superoxide binding to a vacant coordination site on the metal.[20] The copper atom has a sterically blocked axial site facing a hydrophobic region of Cu/Zn SOD, whilst the active site, which is occupied by water in the native protein, faces a narrow hydrophilic channel. The degree of distortion at the copper site is influenced by the positions of the histidines in the protein, and this has a direct influence on the size of the channel leading to the active site.[20] Such structures, accurately determined by X-ray diffraction of single crystals, are not necessarily preserved in solution, the native physical state of superoxide dismutase. In this respect, spectroscopic characterisation of the copper environment by EPR and UV/Vis spectroscopy produces specific information on conformations that should be associated with the biologically relevant structures. Therefore, we constructed a database of previously reported Cu^{II} SODm, by taking into account the hyperfine coupling constants from EPR spectroscopy and the d–d transitions from electronic spectra, as parameters characterising the first coordination sphere around the metal. In addition, we calculated the ratio g_{zz}/A_{zz}, known as distortion factor, f, in order to study in more detail the various distortions of the geometry around the metal expected to be relevant for biological activity. As f indicates the distortions from an "ideal" square-planar geometry formed by the four-coordinate sphere around the metal,[17] we extended its use to a five-coordinate sphere around the metal by assuming that the base of the pyramid has an almost square-planar geometry and can exhibit various distortions with respect to the perpendicular axis where the fifth nucleus is located. It should also be noted that both ^{63}Cu and ^{65}Cu isotopes contribute to the EPR spectra. Although these individual contributions are often not resolved, the observed hyperfine structure represents a weighted average of the parameters from the two isotopes. In contrast to other metals, such as Mn or Ni found in other families of SOD and also used as metal centres in SODm,[7] the sensitivity of the EPR spin Hamiltonian parameters to the coordination sphere geometry in Cu^{II} provides the possibility of using these values for a fine structure–activity screening.

We completed the database with redox potentials and SOD activity values as determined with the indirect method, first reported by McCord and Fridovich.[4b] Even though there are inherent problems in indirect assays,[15a] they represent the conventional way to test for SOD-like activity. As activity parameter, we used the concentration of the copper complex required to attain 50% inhibition of nitro blue tetrazolium (NBT) reduction, the IC_{50} value, as it is indicative of the ability to catalyse superoxide radical dismutation. Various classes of ligands, such as imidazoles, amines, imines, sulfonamides, peptides, salicylates, pyridines and combinations of them, have been included in the database (Table S1, Supporting Information).

Because of the large distribution of IC_{50} values (ca. 0.1 μM to above 200 μM) that have been reported for copper complexes proposed as SODm, we sorted the compounds into two clusters: "active" complexes ($IC_{50} \leq 0.7$ μM) and "inactive" ones (0.7 μM $< IC_{50} <$ 200 μM). We chose the cluster of active SODm to be within about one order of magnitude of that of the native SOD activity, whose value was reported to be between 0.07 μM (horseradish SOD),[10b] and 0.04 μM (bovine erythrocyte SOD).[8e]

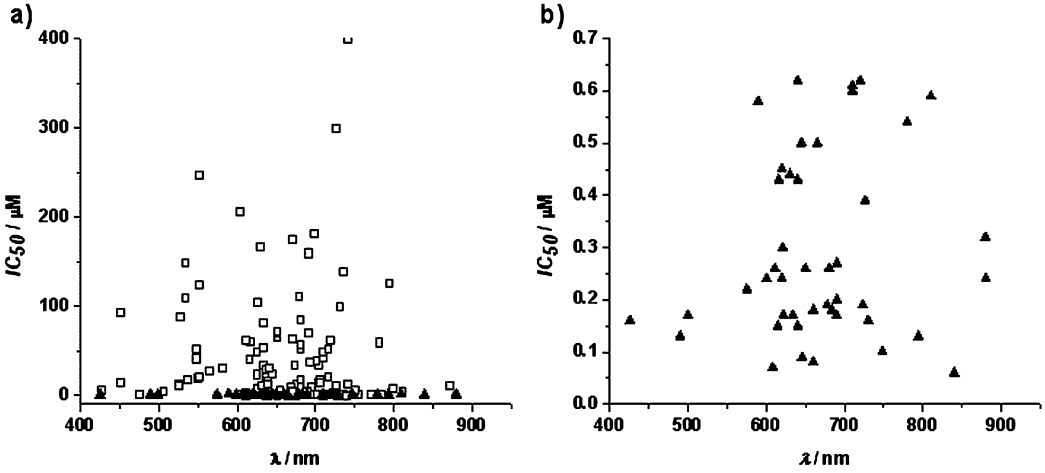

Figure 1. SOD-like activity as a function of the maxima of the d–d transitions in the electronic spectra (λ) for all classes of SODm (a), together with a close look containing only the active compounds (b).

The group of inactive compounds is far larger than that of active compounds, due presumably to the difficulty in attaining the basic requisites of functional SODm.

We first analysed the variation of IC_{50} values as a function of individual spectroscopic parameters, including the wavelength of d–d transitions in UV/Vis spectra (λ), the *z*-component of the hyperfine interaction (A_{zz}) and the distortion factor, *f*, obtained form the EPR spectra. A large distribution of activity values characterises both the whole group of reported complexes and the cluster of highly active ones without any specificity that could involve a characteristic trend (Figure 1 and Figures S1 and S2 in the Supporting Information). We expanded the analysis to consider activity and combinations of structural parameters: λ & A_{zz}, A_{zz} & *f* and λ & *f* for highly active and inactive SODm (Figure 2 and Figure S3 in the Supporting Information). Combining the electronic transition energies with each of the EPR parameters did not yield a specific trend that could distinguish between active and inactive compounds (Figure 2a, b and Figure S3 in the Supporting Information). In contrast, the 3D graph combining the EPR parameters with the IC_{50} values showed an almost linear relationship between EPR parameters for the active complexes (Figure 2d). There was no equivalent relationship for the inactive complexes (Figure 2c).

We refined the analysis of the cluster of active compounds to include a function of copper coordination number (Figure 3a). Both four- and five-coordinate complexes show an almost linear relationships between EPR parameters characterising the coordination sphere around the metal (Figure 3b), and each can be fitted by a linear regression (Table 1).

The overall EPR data show that the cluster of complexes with a four-coordinate sphere around the metal contains the majority of the active SODm compounds, and a slightly distorted square-planar geometry seems to favour high catalytic activity, in agreement with the geometry of native SOD.[20] For example, when we pick two different SODm with the same g_{zz} value (**1** and **2** in Table 2), the more active compound is the one with the smaller A_{zz} value. A decrease

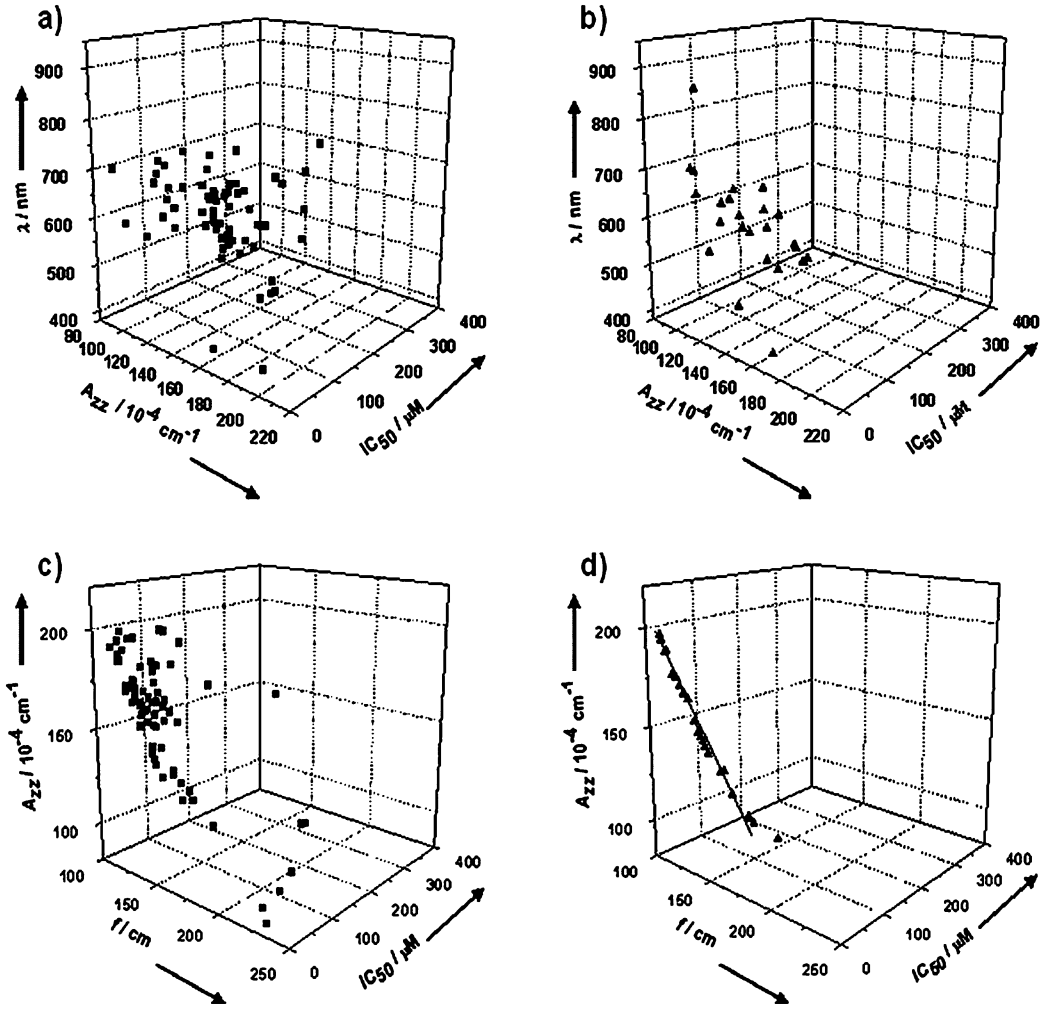

Figure 2. SOD-like activity as a function of the d–d transitions in electronic spectra (λ) and the parallel hyperfine coupling constants (A_{zz}) for the inactive compounds (a) and the active compounds (b). SOD-like activity as a function of the parallel hyperfine coupling constants (A_{zz}) and the *f* factor for the inactive compounds (c) and the active compounds (d).

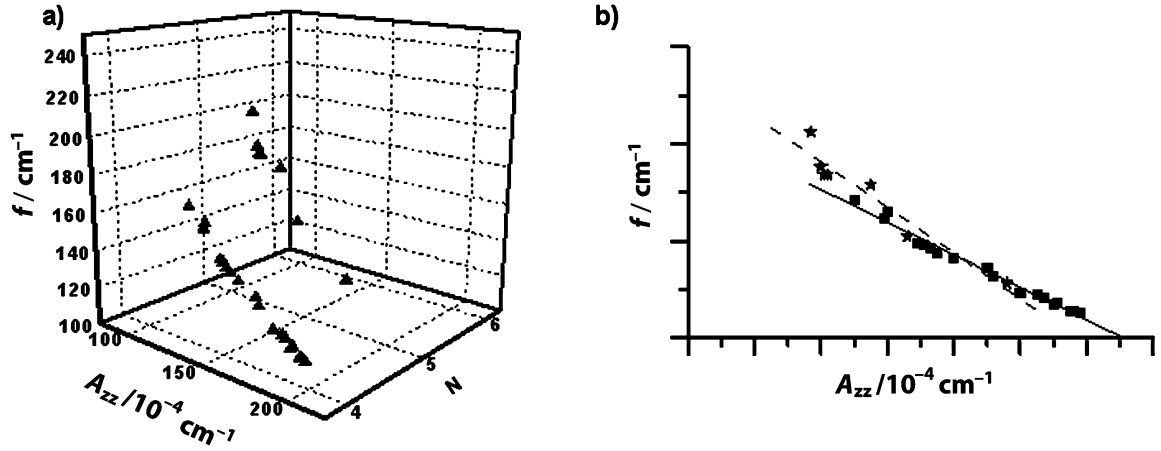

Figure 3. 3D representation of the parallel hyperfine coupling constants (A_{zz}), the f factor and coordination number of copper, N, for active SODm (a). Hyperfine coupling constants (A_{zz}) as a function of the f factor for active SODm (b). Stars: five-coordinate copper complexes; squares: four-coordinate copper complexes.

Table 1. Parameters of the linear regression fit of the f factor as a function of the hyperfine coupling constant, A_{zz} ($f = a + b \times A_{zz}$).

Metal sphere	a	b	r[a]	σ_D[b]
Four-coordinate	277.2 ± 2.5	–0.84 ± 0.03	0.992	2.46
Five-coordinate	331.7 ± 20.3	–1.18 ± 0.15	0.961	7.83

[a] Correlation factor. [b] Standard deviation.

in A_{zz} indicates a tetrahedral distortion of the coordination sphere of the copper atom when coordinated to the same type of atom.[21] Thus, a slight tetrahedral distortion favours the SOD-like activity, as in the native SOD.[20]

Table 2. EPR parameters and SOD-like activity for highly active four-coordinate SODm (Figure 2b) compared with inactive ones.

SODm	A_{zz} [10^{-4} cm^{-1}]	g_{zz}	IC50 [μM]	N	Ref.
1[a]	191	2.248	0.62	4	[8c]
2[b]	139	2.248	0.40	4	[13a]
3[c]	181	2.200	68.0	4	[9d]
4[d]	166	2.225	63.0	4	[8f]

[a] [Cu(dpbi)]$^{2+}$, {dpbi = 2,3-Bis(*N*,*N*′,*N*′′,*N*′′′-2-benzimidazol-2-ylphenylimino)butane}. [b] [Cu(dac-apbi)]$^{2+}$, {dac-apbi = diacetyl-2-(2-aminophenyl)benzimidazole}. [c] [Cu(PMDT)(OAc)]$^{+}$, (PMDT = *N*,*N*,*N*′,*N*′′-pentamethylethylentriamine). [d] [Cu(SAA)(H_2O)], (SAA = salicylidenanthranilic acid).

The number of active complexes with a five-coordinate sphere is significantly smaller than that of those with a four-coordinate sphere, but this could be due to a lack of complete spectroscopic data for other five-coordinate complexes, which could therefore not be included in our analysis. In five-coordinate SODm, the structure of the first coordination sphere around copper is square-pyramidal, with a slightly distorted basal plane. A decrease in the IC_{50} values for decreasing A_{zz} was also found for five-coordinate SODm, although the change in A_{zz} is not as significant as that for four-coordinate complexes, because of geometry restrictions imposed by a pyramidal geometry (**1** and **2** in Table S2, Supporting Information). There were no active complexes with six-coordinate geometries, as expected, indicating that a geometric specificity for an active SODm compound is essential.

We have found that the majority of the SODm are mononuclear irrespective of coordination number and that a binuclear compound is unnecessary for high SOD activity. This is in contrast to literature reports, which claim that a binuclear structure is important for mimicking the SOD enzyme.[11c] Furthermore, biological assays indicate that the role of Zn in native SOD is more related to the stability of the metal binding region than to the catalytic activity.[20b]

We extended our analysis by including the redox potentials of SODm, whose values should be between –0.16 V vs. NHE (for $O_2/O_2^{\cdot-}$) and 0.89 V vs. NHE (for $O_2^{\cdot-}/H_2O_2$) for effective catalysis of superoxide anions. All of the highly active SODm have redox potential values in this range. Contradictory reports concerning the relationship between redox potential and SOD-like activity exist: for a few classes, a linear relationship was reported,[10e,19b] whereas for others, no relationship was found.[8q,9a] By analysing all active SODm whose redox potentials were measured, we found no relationship connecting them to the biological activity. To understand why various proposed SODm were not active, we combined the geometrical condition from EPR data with that of redox potential domain that allows catalysis of superoxide anions. A significant decrease in activity was found both for compounds with distorted geometry, even if their redox potentials were in the appropriate domain for catalytic activity (**3** in Table 2),[9a,9e,9d] and for those with a geometry similar to that of the active ones but with redox potentials outside this domain (**4** in Table 2).[8f]

Our database allows us to distinguish between "promising" ligand classes, such as imidazoles, sulfonamides or peptides, where many proposed complexes are active, and other classes, such as imine, amine and pyridine derivatives, which produced many more nonactive complexes. However, since every ligand class contains both active and inactive

compounds, we consider that the structural conformation at the metal site combined with appropriate redox properties describes the relevant model for highly active SODm.

In conclusion, we have demonstrated the first successful global structure–activity analysis based on all classes of compounds proposed to have a specific biological activity. Contrary to QSAR, we took into account all copper-based SODm, whose electronic and EPR parameters have been published, and established the specificity of the geometry around the metal as an essential criterion for a highly active SODm. Combined with electrochemical properties that favour an effective catalysis, our aim is to optimise further the process of drug discovery. Combinations of spectroscopic and electrochemical properties will be further investigated in the development of our understanding of general features relevant for the biological activity of SODm.

Supporting Information (see footnote on the first page of this article): SOD-like activity as a function of single EPR parameters (Figures S1 and S2); SOD-like activity as a function of the combination of spectroscopic parameters (Figure S3); SODm database (Table S1); EPR parameters and SOD-like activity for highly active five-coordinate SODm (Table S2).

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