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Correlation between the SOD-like activity of hexacoordinate iron(II) complexes and their Fe^{3+}/Fe^{2+} redox potentials

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ABSTRACT

Hexacoordinate, high-spin iron(II) complexes with different isoindoline-derivative ligands have been prepared and characterized. The Fe^{3+}/Fe^{2+} redox transition is reversible in each case, but the $E_{1/2}$ values vary in a ~400 mV range depending on the ligand. SOD-like activity of the complexes was determined by indirect methods with cytochrome c, and nitroblue tetrazolium indicator at pH 7.6. The measured activities correlate with the redox potentials for the Fe^{3+}/Fe^{2+} couples. The results indicate that the superoxide dismutation takes place via an inner-sphere mechanism at the iron site.

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Superoxide dismutase (SOD) enzymes [1] act as a primary defense system against oxidative damage in living species by accelerating the dismutation of superoxide during the catalytic cycle shown in Eq. (1).



Life forms exhibiting SOD activity contain this structurally versatile enzyme with various metal cofactors: Fe [2], Cu/Zn [3], Mn [4] and NiSODs [5] are known. Permanent malfunction of such enzymes may contribute to chronic diseases such as Alzheimer's, Parkinson's, or inflammations, due to overexposing the cells to superoxide and to secondary reactive oxygen species derived from superoxide [6]. It is widely acknowledged, that the redox potential of the metal cofactor is an important determinant of the SOD activity. However, only some studies focus on systematically modified ligand series which are applied to fine-tune the redox properties of the chelated metal ion while providing unchanged stability for the complexes [7]. Here we report the characterization of some iron(II) complexes with a series of

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iminoisoindoline-derivative 3N-donor ligands (Scheme 1) and their SOD-like activity with respect to their redox chemistry. The ligands in Scheme 1 were chosen because of their strong chelating ability (see discussion later) and uncomplicated synthesis. To be noted, that these isoindoline-derivatives show eye-catching similarities with the very popular porphyrins, too: the 3N-donor set is planar, resembling the 3/4 part of a full porphyrin ring, they exhibit informative electronic spectra that are rich in bands, and the extended π -delocalisation within the molecule makes them relatively resistant to chemical changes.

The known 1,3-bis(2'-pyridylimino)isoindoline [8] (HL₂, Scheme 1) has been widely used in enzyme models with copper [9], manganese [10], cobalt [11] and iron [12]. We synthesized derivatives with various side arms on the imine moiety (HL₁, HL₃-HL₅) [13]. For HL₂, two ways of metal ion chelation have been demonstrated. It may act as a tridentate ligand in its neutral [14], or in its deprotonated, anionic form [12,15], due to tautomerism according to Scheme 1. It may be presumed as a general feature for each derivative presented here. In the free ligands, however, the tautomeric equilibrium is shifted toward the isomer, in which the dissociable proton is located on the isoindoline nitrogen. Indeed, as it is seen on the structure of HL₄ (Fig. 1, Table 1) [16], strong interactions between N1, N3, N6 and H1 stabilise the symmetric diimine isomer, thus the molecule adopts planar geometry in the crystalline form.

The bond distances between the carbon and nitrogen atoms are typical for a conjugated π -system. The double bonds are found between the C1–N2 and C8–N5 atom pairs (~1.29 Å) in the diimine-isoindoline moiety, and between the C9–N3 and C17–N6 atom pairs

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Scheme 1. Tautomerism of the ligands and the possible metal chelating modes.

(~1.32 Å) in the benzimidazole heterocycles. Due to the extended conjugation of the π -bonds within the molecule, the UV-vis absorption spectra of the ligands exhibit high intensity, and relatively low energy, multiple π - π * bands in the 360–500 nm region (Table 2).

The bis-chelate complexes $[Fe^{II}(L_1)_2]$ (1), $[Fe^{II}(L_3)_2]$ (3), $[Fe^{II}(L_4)_2]$ (4), $[Fe^{II}(L_5)_2]$ (5) reported here, bearing octahedral geometry were prepared according to Eq. (2), analogously to a reported procedure for $[Fe^{II}(L_2)_2]$ (2) [12,18].

2HL + Fe^{II}(CIO₄)₂.xH₂O + 2Et₃N
$$\xrightarrow{\text{MeOH, Ar}}$$
 [Fe^{II}(L)₂] (2)

The UV–vis absorption maxima of **1–5** (Table 2) that are found between 350 and 460 nm are attributed to the π – π * transitions of the



Fig. 1. Molecular structure of HL₄. Ellipsoids are plotted at the 50% probability. Selected bond distances (Å) and angles (°): C1–N1 1.3843(18), C8–N1 1.3800(19), C1–N2 1.2906(19), C8–N5 1.2939(19), C9–N2 1.3767(19), C17–N5 1.3799(18), C17–N6 1.3235 (18), C9–N3 1.3264(18), N3–H1 2.106, N6–H1 2.112, N1–C1–N2 173.68(6), C1–N1–C8 85.80(6), N1–C8–N5 90.77(7), C1–N2–C9 169.62(6), C8–N5–C17 98.60(7), N1–H1–N6 122.20, and N1–H1–N3 122.29.

coordinated ligands, whereas lower energy bands between 440 and 500 nm, are tentatively rendered to charge transfer transitions from the iron(II) ion to the ligand. In the infrared region the free isoindoline ligands exhibit intense bands in the 1600–1660 cm⁻¹ region that can be assigned as coupled $v_{C=N}$ vibrations. Complexes containing the deprotonated ligands on the other hand show weak band(s) above 1600 cm⁻¹ and stronger ones between 1600 and 1500 cm⁻¹.

The one-electron oxidized analogue of **1**, $[Fe^{III}(L_1)_2](CF_3SO_3)$ -CH₃CN ($\mathbf{1_{ox}}$ ·CH₃CN) was synthesized under dioxygen atmosphere [18] in order to compare the SOD-like activity of the iron(II) and iron (III) forms (*vide infra*). Besides the spectroscopic characterization, Xray structural analysis of the compound was also accomplished. The structure of $\mathbf{1_{ox}}$ (Fig. 2a) shows an almost distortion-free octahedral geometry of the six nitrogen donor atoms around the iron(III) center. The two N_{ind} atoms (N1 and N1A) are situated in the *trans* position to each other, closer to the iron center than the thiazolyl nitrogens by ~0.05 Å. The planes of the coordinated ligand moieties are nearly perpendicular to each other. The Fe–N bond distances in $\mathbf{1_{ox}}$ (~1.95 and 2.00 Å) are shorter than in the analogous iron(II) complexes (2.07 and 2.27 Å in avg. for **2**) [12], and (2.05 and 2.15 Å in avg. for **4**).

The iron(II) complex, **4** was also structurally characterized (Fig. 2b). The two Fe–N_{ind} bond distances (Fe1–N1, 2.0568(17) and Fe1–N1A, 2.0529(17)) are close to the corresponding values for **2** (avg. 2.07 Å). The hexacoordinate iron(II) resides in the center of a strongly distorted octahedron in contrast with that of **2**, or **1**_{ox}. In the structure of **4**, a helical twist of the two ligand planes is seen, causing a significant distortion of the octahedral geometry. This occurs, because the shorter Fe–N_{bim} distances compared to the Fe–N_{py} distances in **2** (the difference is ~0.12 Å) bring the aromatic hydrogens in the 5′-position of the other ligand, and the steric hinderance impedes the settle of an undistorted geometry.

Electrochemical properties of the complex series were investigated with cyclic voltammetry in DMF solution. All compounds show one reversible, one-electron redox wave in the plotted potential range (Fig. 3). This is attributed to the Fe^{3+}/Fe^{2+} redox transition, and spans a 423 mV potential range from 1 to 5 (Table 3). To make a compound thermodynamically competent in the SOD-like reaction, the redox potential of the Fe^{3+}/Fe^{2+} couple should be, as encountered in SODs, between the potential of the two couples O_2/O_2^- and O_2^-/H_2O_2 that is, at pH = 7, -0.40 V and +0.65 V vs. SCE, respectively [7c]. All $E_{1/2}$ values for the iron(II) complexes fall into this range. The process is reversible with very similar E_a and E_c peak separations for each complex (~100 mV), similarly to the ferrocene internal standard. Although a possible explanation for this relatively wide potential range could be the change of the spin-state of the iron(II) ion depending on the coordinating ligand, based on the available Mössbauer data for the two structurally characterized complexes, 2 [12] and **4** (zero field isomer shifts are 1.018 and 1.12 mms⁻¹, and quadrupole splittings are 1.975 and 1.32 mms⁻¹ at 80 K, respectively), these iron(II) complexes are high-spin. Assuming that one of the two e_g electrons is removed during the oxidation step, the relative energy of these electrons throughout the series can be the governing factor for the changes in the $E_{1/2}$. The relative energy of the e_g set should follow the tendencies in the ligand-field strength, as a result the observed increase in the $E_{1/2}$ from **1** to **5** allows us to suppose a decrease in the ligand-field. In other words, the stronger the ligandfield effect, the easier it is to oxidize the iron(II) center. The notable difference between the bond distances measured for 2 and 4 is in accordance with this explanation.

Reactivity of **1–5** and **1**_{ox} with superoxide anion was investigated in aqueous HEPES buffer following the modified McCord–Fridovich assay [19] in the presence of catalase enzyme [20]. Complexes **1–5** and **1**_{ox} inhibit the reduction of NBT [21]. Since the *IC*₅₀ concentrations are proportional to the concentration and type of indicator, we calculated the apparent k_{cat} rate constants as $k_{cat} = k_I [1]/IC_{50}$ (where k_I is 5.94×10⁴ M⁻¹ s⁻¹, when I = NBT

Table 1							
Crystal	structure	details	for	1 _{ox} ·	CH ₃ CN,	4 and	HL ₄ .

	$1_{ox} \cdot CH_3CN$	4	HL ₄
Chemical formula	$C_{13}H_{19}F_3FeN_{11}O_3S_5$	C48H35F3FeN14	$C_{24}H_{19}N_3$
Formula weight	866.77	863.75	405.46
Space group	Manoclinic, P 21/c	Triclinic, P-1	Monoclinic, P 21/n
a, Å	14.0476(14)	13.0522(19)	8.4949(2)
b, Å	20.5817(17)	14.261(3)	16.9348(3)
<i>c</i> , Å	12.2870(10)	14.691(2)	13.946(3)
α , deg	90	63.983(6)	90
β , deg	103.000(2)	74.430(6)	95.758(1)
γ, deg	90	70.724(6)	90
V, Å ³	3461.4(5)	2294.9(7)	1996.24(7)
Z	4	2	4
$D_{\rm calc}$, mg m ⁻³	1.663	1.250	1.349
Temperature, K	296(2)	293(2)	203(2)
Unique reflections	7926	10,065	5085
data> 2 σ parameters/ restraints	4999/507/0	7964/572/0	3818/282/0
$R_1^{a} [F2 > 2\sigma (F2)],$ $wR_2^{b} (F2)$	0.0478, 0. 1060	0.0495, 0.1377	0.0496, 0.1125
Goodness of fit	1.030	1.043	1.083

^a $R_1 = \left(\sum ||F_o| - |F_c|| \right) / \left(\sum |F_o| \right).$ ^b $wR_2 = \left[\sum w(F_o^2 - F_c^2)^2 \sum w(F_o^2)^2 \right]^{1/2}, w = 1/\sigma^2(Fo^2) + (AP)^2 + BP, \text{ where } P[F_o^2 + 2P_o^2]$ $(F_o^2 + 2(F_c^2))/3$, A = 0.0562, 0.0978 and 0.0532, B = 0, 0 and 0.7956 for **1**_{ox}.CH₃CN, **4** and HL₄, respectively.

[22] and 2.0×10^5 M⁻¹ s⁻¹ when I = cytochrome c [23]) for comparisons with the literature data (Table 3). The $[Fe^{II}(L_n)_2]$ complexes show activities comparable to those of iron(II) species containing TPEN-derivatives [7a]. Although positive indirect tests applied here do not provide conclusive evidence on the real catalytic activity of putative SOD mimics, testing both the oxidized and reduced forms is a good indication that the complexes can indeed act as catalysts in the dismutation of superoxide anion. With $\mathbf{1}_{ox}$ which is the iron(III) analogue of 1 similar activity was measured with that of 1 (Table 3). Therefore we propose, that these complexes may be considered as catalysts of superoxide anion dismutation.

The determined k_{cat} (also the IC_{50} values) for **1–5** correlate with the observed $E_{Fe}^{3+}_{/Fe}^{2+}$ potentials [24] (Fig. 4). Similar correlations with a positive slope have been described previously for manganese(III)- and iron(III)-porphyrins [7]. Increase in the SOD-like activity with the redox potential becomes explainable if one assumes that the reduction of the

Table 2

UV-vis absorption data of	of the free ligands and th	e complexes 1–5 in DMF
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Ligand	$\pi - \pi^*$ CT bands of the free ligands λ_{max}/nm (log ε)	$[Fe(L)_2] \lambda_{max}/nm \ (\log \varepsilon)$	Ref.
HL ₁	485 (3.18)	788 (4.01)	
	448 (3.86)	463 (4.43)	
	420 (4.05)	435 (4.50)	This work
	396 (4.01)	404 (4.53)	
	374 (3.94)	346 (4.30)	
HL ₂	410 (3.99)	440 (4.58)	
	386 (4.23)	410 (4.81)	14 ^a
	366 (4.19)	390 (4.75)	12 ^b
HL ₃	408 (4.11)	439 (4.50)	
	385 (4.29)	411 (4.65)	8 ^a
	366 (4.24)	392 (4.57)	This work ^b
HL_4	478 (4.11)	487 (4.45)	
	447 (4.35)	454 (4.62)	
	420 (4.33)	433 (4.60)	This work
	393 (4.38)	349 (4.55)	
	373 (4.39)		
HL ₅	470 (4.06)	493 (4.28)	
	440 (4.30)	453 (4.49)	10 ^a
	413 (4.31)	389 (4.40)	This work ^b
	388 (4.35)	367 (4.45)	
	369 (4.37)	• •	

^a Ligand.

^b Complex.



Fig. 2. Molecular structures of a) 1_{ox}·CH₃CN and b) 4. Selected bond distances (Å) and angles (°) for 1ox · CH₃CN: Fe1-N1 1.946(2), Fe1-N1A 1.952(2), Fe1-N2 2.003(2), Fe1-N2A 2.005(2), Fe1-N3 2.003(2), Fe1-N3A 1.997(2), N1-Fe1-N1A 178.70(10), N2-Fe1-N3 175.99(10), N3A-Fe1-N2A 177.98(10), N1-Fe1-N2 89.23(9), N2-Fe1-N2A 92.06(9), N1-Fe1-N2A 89.62(9); and for 4: Fe1-N1 2.0568(17), Fe1-N1A 2.0529(17), Fe1-N4 2.1356 (17), Fe1-N4A 2.1471(17), Fe1-N6 2.1296(17), Fe1-N6A 2.1318(17), N1-Fe1-N1A 173.68 (6), N1-Fe1-N4 85.80(6), N1-Fe1-N6 90.77(7), N6-Fe1-N6A 169.62(6), N1A-Fe1-N4 98.60(7), N4-Fe1-N4A 168.40(7). Solvent molecules and hydrogen atoms are removed for clarity. The CF₃SO₃⁻ counterion and some ligands are plotted as spheres for viewing purposes. Ellipsoids are plotted at the 50% probability.

iron(III) form is the rate-limiting step. On the basis of our results, discerning between an inner-sphere, or an outer-sphere redox mechanism is uncertain. However, in the case of the latter, in which superoxide anion does not coordinate to the metal center, one would expect that those complexes will show higher activity, which have redox potentials close to the midway value between the two potential values for the superoxide anion as a result of an equal thermodynamic driving force for both the reduction and the oxidation steps [7f]. In addition, it was shown for monohydroxoiron(III) and aquomanganese (III) porphyrins that in case of an outer-sphere mechanism, a 120 mV increase in $E_{1/2}$ imparts a roughly 10-fold increase in k_{cat} , in accordance



Fig. 3. CVs of compounds **1–5** (0.5–1.0 mM) taken at a scan rate of 100 mVs⁻¹ using a GCE working electrode, Pt-wire auxiliary electrode, and a Ag/AgCl reference electrode in DMF. Potentials are referenced to the ferrocene/ferrocenium couple.

with the Marcus equation for outer-sphere electron-transfer reactions [7b]. With our complexes the observed change in k_{cat} is only 30% per ~120 mV. Based on these considerations, we think the inner-sphere mechanism is more plausible for the redox steps according to Eq. (1) for **1–5**. To further elucidate this question, thiocyanate was added to a solution of compound **2** in 5:1 ratio, because this small ligand was investigated in heptacoordinate iron(II) complexes earlier [25]. In the UV–vis absorption spectrum of the mixture a new band occurs at 460 nm [26] besides the unchanged bands of **2** indicating the presence of a heptacoordinate species. This emphasizes that an additional small, anionic ligand like SCN⁻ may add to the iron(II) center.

Cross-reactions (that could modify the observed IC_{50} values) between the redox indicator NBT and the tested high-spin iron complexes could be excluded based on our experiments. Therefore the observed linear correlation between the redox potential of the complexes and their SOD-like activity indicates that the rate determining step in the superoxide dismutation is the reduction of the metal center. Formation of a heptacoordinate transition state by the addition of superoxide to the octahedral complexes is a plausible mechanism based on our tests with SCN⁻.

Table 3

Apparent rate constants and redox potentials of iron complexes exhibiting SOD-like activity.

Comp1ex ^a	$k_{\rm cat} \ (10^6 {\rm M}^{-1} {\rm s}^{-1})^{\rm b}$	$E^{\circ\prime c}_{ox/red}$ (V)	Ref.
1	$5.00 \pm 0.35\;(4.62 \pm 0.32)$	0.555	This work
1 _{ox}	5.28 ± 0.30		
2	3.83 ± 0.20	0.319	This work
3	3.62 ± 0.20	0.265	This work
4	$3.06 \pm 0.15\;(3.28 \pm 0.15)$	0.205	This work
5	$2.50 \pm 0.22 (2.26 \pm 0.24)$	0.123	This work
FeCl ₂	0.21 ± 0.05		This work
[Fe ^{III} BIG)Cl ₂]	0.041		[7c]
[Fe ^{II} (TPAA]	2.15		[24]
[Fe ^{II} (6MeTPEN)] ²⁺	3.5	0.640	[7a]
[Fe ^{II} (TPEN] ²⁺	14.0	0.590	[7a]
$[Fe^{II}((4Me)_4TPEN)]^{2+}$	35.0	0.510	[7a]
[Fe ^{II} ((4MeO) ₄ TPEN] ²⁺	70.0	0.425	[7a]
Fe-SOD	~220 ^d	0.020	[27]
Cu,Zn-SOD	~2000 ^d	0.160	[6]
cob(II)alamin	700		[28]

^aLigand abbreviations: BIG, N,N-bis[1-methyl-2-imidazolyl)methyl] glycinate TPAA. tris[2-[N-(2-pyridylmethyl)amino]ethyl]amine; 6MeTPEN, N-(6-methyl-2-pyridy1emrthyl)-N,N', N'-(2-pyridy1methyl)ethylenediamine; (4Me0)₄TPEN, N,N,N',N'-(2-pyridy1methyl) ethylenediamine; (4Me0)₄TPEN, N,N,N',N'-(4-methoxy-2-pyridy1methyl)ethylenediamine; (4Me0)₄TPEN, N,N,N',N'-(4-methoxy-2-pyridy1methyl)ethylenediamine; bValues in parenthesis are from the cytochrome assays.^cvs. SCE. d_{kcat}/K_m values.



Fig. 4. Dependence of the measured SOD-like activity of **1–5** on the $E_{Fe^{3+}/Fe^{2+}}^{\omega}$ values. (Potentials are plotted vs. SCE. $E_{Fe^+/Fc}$ vs. SCE was determined experimentally to be $+500 \pm 10$ mV in our system and was added to the experimentally determined $E_{Fe^{3+}/Fe^{2+}}^{\omega}$ values to gain the above plot.)

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Appendix A. Supplementary material

Files contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/ cif.

Supplementary data to this article can be found online at doi:10.1016/j.inoche.2010.10.023.

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- [13] 1. 3-bis(2'-pyridylimino)isoindoline (HL₂) [8], 1. 3-bis(4'-methyl-2'-pyridylimino) isoindoline (HL₃) [8] and 1, 3-bis(2'-benzimdazolylimino)isoindoline (HL₅) [10] were prepared according to published procedures. 1,3-bis(2'thiazolylimino)isoindoline (HL1). Phthalonitrile (3.84 g, 30 mmol) and 6.00 g (60 mmol) 2-aminothiazole were melted at 180 °C and stirred vigorously for 9 hours, until ammonia evolution ceased. The product was cooled to room temperature and diethyl ether was added (100 mL). The dark brown crystals of the product were filtered and vacuum dried. The crude ligand was recrystallized from a minimal amount of boiling N. N-dimethylformamide. Yield: 7.44 g (80%). M.p.: 258–260 °C. Anal. Calcd for C₁₄H₉N₅S₂: C, 54.00; H, 2.91; N, 22.49. Found: C, 54.30; H, 2.82; N, 22.32. FT-IR bands (KBr pellet, cm⁻¹) 3207(w), 3109(w), 3076 (w), 1622(s), 1478(m), 1376(w), 1299(m), 1213(s), 1131(s) 1050(m), 873(w), (3.86), 420 (4.05), 396 (4.01), 374 (3.94), 288 (3.54). ¹H-NMR (CDCl₃), 8/pm: 8.00 (dd, 2H, J₁ = 5.8 Hz, J₂ = 3.2 Hz), 7.77 (d, 2H, J = 3.3 Hz), 7.63 (dd, 2H, J₁ = 5.8 Hz, J₂ = 3.3 Hz), 7.18 (d, 2H, J = 3.3 Hz), 13 C-NMR (CDCl₃), δ /ppm: 117.6, 122.9, 132, 135, 141, 152.7, 171.8. 1,3-bis(1'-methyl-2'-benzimidazolylimino) isoindoline (HL₄). This ligand was synthesized as it was reported for HL5 earlier [9], Yield: 85%. M.p.: 197–199 °C. Anal. Calcd for C₂₄H₁₉N₇: C, 71.09; H, 4.72; N, 24.18. Found: C, 70.92; H, 4.83; N, 24.32. FT-IR bands (KBr pellet, cm⁻¹) 3111(w), 3048(w), 2957(w), 2924(w), 1621(s), 1478(m), 1434(m), 1321(m), 1266(m), 1209(s), 1095(s) 1050(m), 824(w), 739 (s), 694(w). UV-vis (DMF) [λ_{max}, nm (logɛ)] 478 (4.11), 447 (4.35), 420 (4.33), 393 (4.38), 373 (4.39), 348 (4.33), 316 (4.19), 289 (4.37). ¹H-NMR (CDCl₃), δ/ppm: 8.23 (m, 4H), 7.90 (m, 4H), 7.62 (m, 4H), 4.06 (s, 6H).
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- [16] The crystal evaluations and intensity data collections were performed either on a Rigaku R-AXIS Rapid single-crystal diffractometer (1_{ox} and 4), or a Bruker-Nonius Kappa CCD single-crystal diffractometer (H_{L_4}) using Mo K α radiation ($\lambda = 0.71070$ Å) at 296, 293, and 203 K, respectively. Crystallographic data and details of the structure determination are given in Table 1, whereas selected bond lengths and angles are listed in the corresponding figure captions. SHELX-97[17] was used for structure solution and full matrix least squares refinement on F^2 . CIF files are available in the CCDC database: 1_{ox} ·CH₃CN (CCDC 756934), **4** (CCDC 756935).
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(w), 1572(s), 1516(s), 1466(s), 1394(m), 1346(w), 1288(m), 1184(m), 1066(s), 996(m), 931(m), 805(m), 706(m). UV-vis (DMF) [λ_{max} , nm (loge)] 439 (4.50), 411 (4.65), 392 (4.57), 347 (4.19), 331 (4.32), 307 (4.43). **4**. Yield: 61%. Anal. Calcd for C₄₈H₃₆FeN₁₄: C, 66.67; H, 4.20; N, 22.68. Found: C, 66.51; H, 4.13; N, 22.72. FT-R bands (KBr pellet, cm⁻¹) 3056(w), 2933(w), 1622(s), 1552(s), 1483 (w), 1471(m), 1434(w), 1386(m), 1325(m), 1287(w), 1186(m) 1099(m), 1074 (m), 739(m). UV-vis (DMF) [λ_{max} , nm (loge)] 487 (sh, 4.45), 454 (4.62), 433 (sh, 4.60), 349 (4.55). Crystals suitable for X-ray structural determination were obtained from DMF. **5**. Yield: 72%. Anal. Calcd for C₄₄H₂₈FeN₁₄: C, 65.35; H, 3.49; N, 24.25. Found: C, 65.19; H, 3.37; N, 24.08. FT-IR bands (KBr pellet, cm⁻¹) 3419(m), 3060(w), 1616(m), 1533(s), 1471(m), 1446(m), 1416(m), 1374(w), 1315(m), 1271(m), 1191(m) 1115(m), 1029(w), 748(m). UV-vis (DMF) [λ_{max} , nm (loge)] 493 (4.28), 453 (4.49), 389 (4.40), 367 (4.45), 319 (3.29).

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- The SOD-like activity of the iron complexes was studied at 298 K by using indirect [20] method of nitroblue tetrazolium (NBT), or cytochrome c reduction. The superoxide radical anion was generated in situ by the xanthine/xanthine oxidase reaction, and detected spectrophotometrically by monitoring the formation of diformazan from NBT at 560 nm, or alternatively, the reduced form of cytochrome c at 550 nm. The test reactions were carried out in 0.01 M HEPES (N-2hydroxyethylpiperazine-N'-2-ethanesulfonic acid) buffer at pH = 7.6. The reaction was initiated by adding an appropriate amount of xanthine oxidase to a stirred solution of NBT $(1.7 \times 10^{-4} \text{ M})$, or cytochrome $(5.2 \times 10^{-5} \text{ M})$, xanthine (10^{-4} M) and catalase enzyme (175 U/3 mL) to generate $\Delta A_{560 \text{ or } 550} = 0.025$ -(10 M) and ratia catalast charging (1750/5 m) to generate an $_{500}$ (1550 m) 0.028 min⁻¹. The NBT reduction rate was measured either in the absence or presence of putative SOD mimics ($0-20 \times 10^{-6}$ M). The possible inhibition of the xanthine oxidase enzyme by the SOD mimics was excluded by monitoring the urate formation at 296 nm in separate test reactions. The SOD-like activity was then characterized by determining the IC_{50} values (μ M, the concentration where inhibition of NBT reduction is 50%) for each compound. The IC_{50} concentrations can be obtained from $\Delta A_0/\Delta A_X - 1$ vs. complex concentration plots as follows: complex concentration, X $[\mu M] = IC_{50}$ where $\Delta A_0 (\Delta A_X - 1 = 1 (\Delta A_0 \text{ is the change in absorbance at 560 nm min⁻¹ in the absence of complex; <math>\Delta A_X$ is the change in absorbance at 560 nm min⁻¹ in the presence of X μM of the complex). Alternatively, IC_{50} can be determined when $100(\Delta A_0 - \Delta A_X)/\Delta A_0$ is plotted against the complex concentration. The data can be fitted with a first order exponential curve, and the 50% inhibition on the curve shows the IC₅₀. The two methods are equally suitable for the determination of the IC50 values.
- [21] In separate test reactions the inhibition of the xanthine/xanthine oxidase system by the iron(II) complexes was excluded. The reduction of NBT by superoxide anion takes place in a one-electron process giving tetrazolinyl radical, that disproportionates to formazan, which gives indicator colour and NBT. Under assay conditions with 1 mM/min superoxide anion production, where the concentration of the complex and NBT is higher than that of the substrate, a mixture of the reduced and oxidised form of the complex is present simultaneously. This way, there is a possibility of cross reaction between the oxidised form of the putative SOD mimic complex, and the tetrazolinyl radical that leads to overestimation of the SOD activity. To elucidate this problem, the SOD-like activity was tested in three cases (1, 2 and 5, Table 4) with cytochrome c indicator, too. No significant difference in the kCat values was found using cytochrome c compared to the results from the NBT assays, therefore the above depicted cross reaction does not interfere with the superoxide scavenging reactions.
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