Recent Advances in the Design of Iron Chelators Against Oxidative Damage

J.-B. Galey*

L'OREAL Recherche Avancée, Department of Chemistry, 1 avenue Eugène Schueller, 93600 Aulnay sous bois, France

Abstract: Iron imbalance plays a pivotal role in oxidative damages associated with a wide range of pathological conditions. However, owing to the essential role of iron in biological processes, the beneficial effects of iron chelation therapy against oxidative damage have to be balanced against potential toxicity. The present review briefly introduces iron redox biochemistry and oxidative-stress associated pathologies, surveys recent advances in iron chelating strategies and summarizes some of our recent findings in this field, with a special emphasis on the chemical design constraints one must satisfy in order to synthesize iron chelators which could be beneficial against oxidative stress without inducing iron depletion of the body. The concept of oxidative stress activatable iron chelators is presented as a new paradigm for safe and efficient treatment of oxidative-stress associated conditions.

INTRODUCTION

Iron fills a central position in biological processes. Thanks to its highly flexible coordination chemistry and redox potential, which can be finely tuned by coordinating ligands, it is critically involved in a variety of cellular events ranging from respiration to DNA synthesis. However, cells have to maintain the concentration of free iron as low as possible so as to avoid its interaction with dioxygen and reduced oxygen species which otherwise could induce toxic effects. Indeed, when not carefully handled by proteins and shielded from surrounding media, iron is a powerful catalyst of uncontrolled oxidative damage to DNA, proteins and lipids. In the presence of physiological reductants, iron can undergo a redox cycling between the two oxidation states, thereby generating continuous production of highly reactive oxygen species [1, 2]. Moreover, since free iron can non-specifically bind to DNA and most proteins, the resulting damage cannot be prevented by radical scavengers, except at very high concentrations, because the oxidizing species reacts very closely to the metal-binding site according to so-called site-specific Fenton reaction as shown in (Fig. **1**) [3, 4].

Therefore, iron levels are tightly controlled in biological systems by transport and storage proteins in order to minimize undesired radical reactions. However, in some situations the iron balance can be disturbed either locally or systemically. In systemic iron overload, excess iron saturates the binding sites of transferrin, allowing free iron to circulate and to catalyse oxidative damage, ultimately leading to heart failure [5, 6]. Systemic iron overload is associated with several diseases such as hereditary haemochromatosis and thalassaemia major. The former requires repetitive blood transfusions which inevitably leads to an excess iron in the

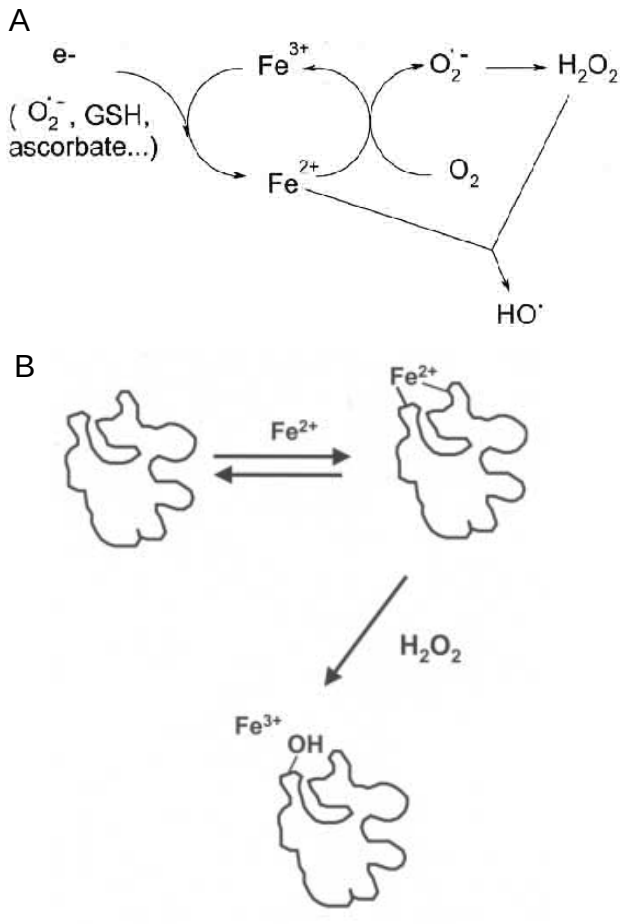

Fig. (1). (a) Reductant-driven Fenton chemistry leading to hydroxyl radical formation. (b) Site specific oxidation catalysed by iron on a macromolecular target which can non specifically bind iron

*Address correspondence to this author L'OREAL Recherche Avancée, Department of Chemistry, 1 avenue Eugène Schueller, 93600 Aulnay sous bois, France; Tel (33) 1 48 68 94 58; Fax (33) 1 48 68 94 89; E-mail: jbgaley@recherche.loreal.com

1389-5575/01 $20.00+.00

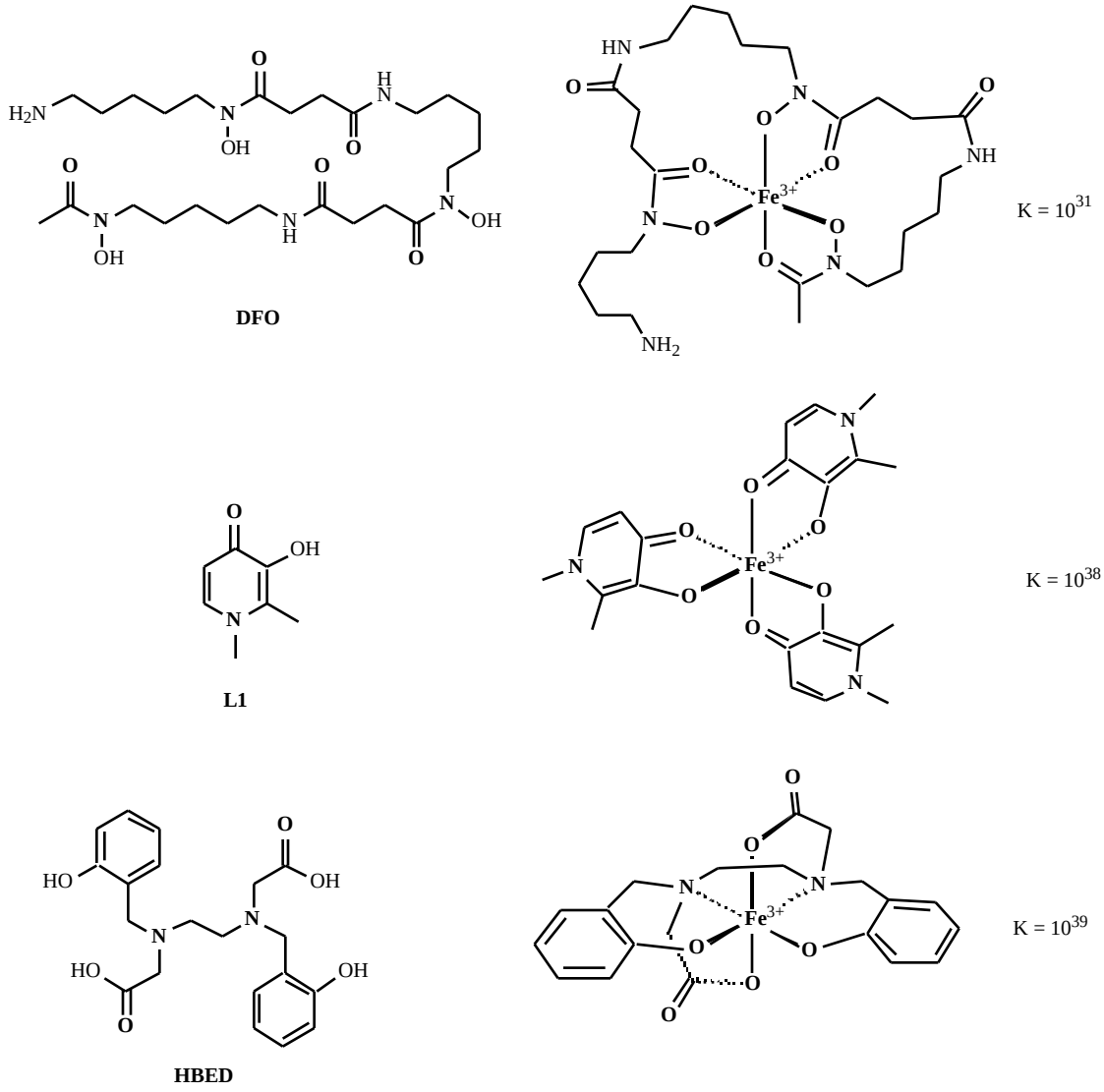

Fig. (2). Structures of main iron chelators discussed and of their corresponding ferric iron complexes with overall stability constants.

body since iron levels are only regulated by absorption. In most of these systemic iron overload situations, treatment by iron chelators such as desferrioxamine (DFO, Fig. **2**) is the only effective way to remove excess iron [7, 8].

On the other hand, in oxidative stress conditions, iron is locally released from its normal storage sites and becomes involved in Fenton chemistry [9, 10]. This paper will focus on the potential use of iron chelators for the treatment of such conditions associated with oxidative damage or local disturbance of iron homeostasis, with a special emphasis on possible side effects related to interactions of the chelators with normal iron metabolism.

The aim of the present mini review is also to provide an overview on iron chelation therapy and more specifically to describe the concept of oxidative stress activatable iron chelators. In order to allow the reader to better understand these points, the first part of the paper tries to condense the basic information in the field of iron redox biochemistry and oxidative stress-associated pathologies.

IRON REDOX BIOCHEMISTRY

Iron level in normal human adults is maintained at approximately 4-5 g, mostly in the form of hemoglobin within red cells. About 10% is contained in myoglobin, cytochromes and other iron-containing enzymes. The remaining 20-30% are distributed between storage protein ferritin and its lysosomal degradation product haemosiderin. Transferrin only accounts for 0.1-0.2% of the total body iron. In humans, the average daily absorption of iron is 1-3 mg/day. About the same amount is lost by cell desquamation, mostly from the gut but also from skin and via urine and bile (see [11] for review). Thus, under normal circumstances, iron is used by the body in an almost closed circuit [12]. Iron is absorbed through the gut and transported to transferrin which has two high affinity binding sites for ferric iron ($K=10^{21}$). Plasma transferrin (about 30 µM) is normally only 20-30% saturated. Thus the concentration of free ferric iron in plasma is usually very low (below 10^{-12} M). This extremely low level of iron also accounts for much of the bacteriostatic effects of human plasma by depriving

microorganisms of iron required for their growth. Cells which require iron for maturation or proliferation express high densities of transferrin receptors on the surface of their plasma membrane. Cellular uptake of iron transferrin then involves internalisation of transferrin receptor complex into an acidic endosome. Within the cytosol, iron is incorporated into ferritin probably as Fe(II) which becomes oxidized within the protein core. Ferritin is normally only 20-30% iron-saturated. Cellular homeostasis of iron is maintained by the coordinate regulation of the expression of transferrin receptor and ferritin at a posttranscriptional level. The expression of these proteins is regulated by intracellular iron level through its interaction with Iron Responsive Proteins (IRPs, see [13] for review).

IRON AND OXIDATIVE STRESS

Despite the remarkable efficacy of iron transport and storage systems, various conditions of oxidative stress are associated with a local release of iron from normal sites. Release of iron from ferritin requires reduction in the presence of a ferrous iron acceptor [14-16]. Physiological reductive agents such as ascorbate and glutathione do not release iron from ferritin at significant rates, but superoxide is an important mediator of its release [17, 18]. Proteolytic degradation of ferritin during oxidative stress is likely to also contribute to iron release [19].

It is important to outline that superoxide toxicity is as well related to inactivation of a number of enzymes containing [4Fe-4S] clusters such as dehydratases and aconitases with concomitant loss of ferrous iron from the cluster [20-22]. In red cells, which are rich in antioxidant enzymes, iron is tightly bound to hemoglobin but injured or lysed red cells may release iron into the surrounding medium. Indeed, hemoglobin has been shown to release iron ions when exposed to excess hydrogen peroxide [23]. Considering the amount of iron in red cells, red cell injury by various mechanisms has therefore the potential to release important amounts of iron at the site of injury and thus to induce oxidative damage. In particular, bleeding as a result of injury could lead to hemoglobin release with a possible compounding effect [24].

IRON-DEPENDENT OXIDATIVE STRESS-ASSOCIATED PATHOLOGIES

Inflammatory Disorders

Infiltrations by neutrophils and macrophages into an inflamed area produce high local levels of superoxide and nitric oxide. This respiratory burst is likely to induce local iron release from ferritin. Degradation of ferritin by the autophagic vacuolar apparatus in macrophages during inflammation is also considered to be responsible for iron release [25]. Synovial fluid of patients with rheumatoid arthritis contains increased amounts of both low molecular weight iron species and ferritin-bound iron. The presence of iron associated to hydrogen peroxide generated by activated inflammatory cells is believed to participate to the degradation of involved tissues. Studies of experimental arthritis in rats showed that iron chelators such as DFO reduced the chronic inflammatory phase although DFO was unable to prevent the acute phase [26]. So far, only limited clinical studies were conducted, mainly with DFO. In DFO-treated patients, serious side effects were observed, including anemia and cerebral toxicity with rather disappointing results [27, 28].

Post-ischaemic Reperfusion Injury

Intracellular free iron increases dramatically during ischemia, probably because of the accumulation of reducing equivalents which arise during ischemia and also because of ischemia-induced acidosis [29]. Such free iron is believed to catalyse the production of a pulse of hydroxyl radicals when oxygen tension is suddenly restored during reperfusion. Therefore, the use of iron chelators has been proposed in order to reduce post-ischaemic tissue damage. A number of studies have shown that DFO improved survival and physiological function in various models of cerebral or cardiac ischemia/reperfusion [30, 31]. A significant protection was also obtained with hydroxypyridinone L1 (Fig. **2**) on isolated rat hearts as measured by contractility after reperfusion [32]. These results suggest that iron chelators could be useful in the treatment of stroke and heart attack. Iron chelation could also be beneficial during cardiopulmonary bypass surgery for which a transient ischemia occurs as well as in organ transplantation by increasing viability of transplanted tissues [33].

21-amino steroids of U-74500A series (Fig. **3**) have provided interesting results in animal model systems of ischemia reperfusion injury to the brain or spinal cord [34]. These drugs were designed as membrane specific antioxidants by attaching a membrane localizing steroid to an antioxidant amine which could either act as lipid peroxyl radical scavenger or as ferrous iron chelator [35, 36]. However, although it can be suspected that U-74500A inhibition of lipid peroxidation is partially related to a modulation of iron redox chemistry, additional mechanisms are likely to occur.

Intoxication By Xenobiotics

Several xenobiotics which can undergo redox cycling have the capacity to generate superoxide and to release iron from ferritin thereby inducing tissue damage [9]. They include toxic herbicide paraquat, antineoplastic drug adriamycin, and diabetes-inducing chemical alloxan. These compounds easily undergo one-electron reduction by NADPH cytochrome P450 reductase to generate a free radical which can either autoxidize, yielding superoxide, or directly induce reductive iron release from ferritin. The cardiotoxicity of anthracyclines such as doxorubicin unfortunately limits their clinical usefulness although they are very effective anticancer drugs. Doxorubicin toxicity is believed to be mediated by redox cycling of iron anthracycline complex, the drug being accumulated in heart tissue. Treatment with ICRF-187 (Dexrazoxane, Fig. **3**) has been shown to protect against doxorubicin cardiotoxicity without disturbing the antitumor effect [37]. This drug acts via its hydrolysis

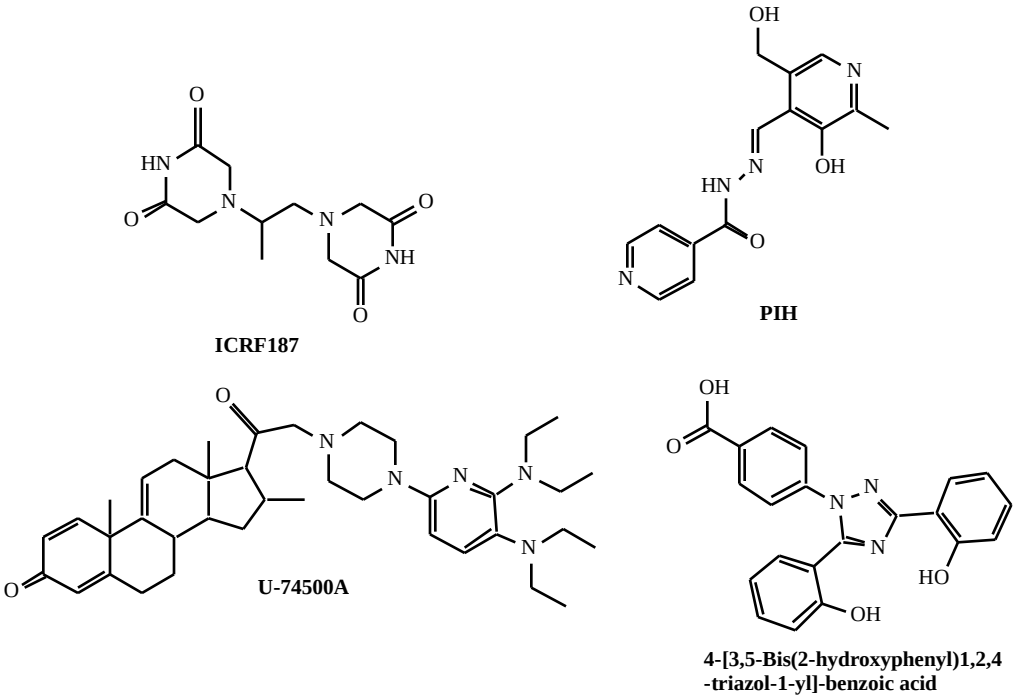

Fig. (3). Structures of other potentially useful iron chelators

product ADR-925, the structure of which is similar to that of EDTA and is therefore able to remove iron from the iron-anthracycline complex. Although iron ADR-925 chelate, like iron-EDTA complex, is a good catalyst of hydroxyl radicals formation (*vide infra*), its overall effects are protective probably because it avoids site-specific damage by orienting damage towards less sensitive targets [38, 39]. Moreover, additional mechanisms involving topoisomerase II inhibition have been recently described as well [37].

Atherosclerosis

Oxidised low-density lipoproteins play an significant role in atherogenesis. The importance of copper and iron in oxidative modifications of LDL increasing their affinity for subendothelial macrophages and their atherogenicity is well known. Gruel samples from advanced atherosclerotic lesions were found to contain significant amounts of bleomycin-detectable iron [40]. However, direct experimental evidence of the involvement of catalytic iron in the generation of atherosclerotic lesions is still lacking as is the origin and form of such iron. Studies were performed in the 1950s and 1960s using EDTA as therapy for atherosclerosis. No significant improvement was observed for EDTA treated patients [41]. However EDTA is not the best candidate to prevent iron dependent oxidative damage for reasons discussed below.

Neurodegenerative Diseases

Several areas of the human brain are rich in iron as protein-bound forms, although cerebrospinal fluid has a low content in transferrin and only moderate amounts of SOD and glutathione peroxidase [42]. Thus, being very rich in polyunsaturated fatty acids, brain is especially prone to iron-dependent oxidative damage. Ischemic or traumatic brain injury, by causing partial homogenization, is thought to be able to increase catalytic iron availability and to induce lipid peroxidation (see [3] for review). Dopamine-rich regions of the brain may be especially sensitive to altered iron metabolism, firstly because monoamine oxidase produces hydrogen peroxide, and secondly because dopamine firmly binds iron and, as a catechol, can undergo redox cycling and produce reduced oxygen species. Although direct demonstration is lacking, evidence suggests that oxidative stress is involved in Parkinson's disease and is associated with the loss of dopaminergic neurons. An increased iron content have been found in substantia nigra of postmortem parkinsonian brain. The cause of this disturbance of iron homeostasis and the physical nature of iron (which could be complexed with neuromelanin) are not known in detail [43-45]. Altered brain metabolism has also been proposed to be linked to Alzheimer's Diseases [46] but it is not known whether the alteration is an early or a late event in the disease process. Potential use of iron chelators has been proposed against neurodegenerative diseases. However, one the major problems remains chelator transport across the blood brain barrier. Intramuscular injection of DFO to patients with Alzheimer's disease was found to have a significant effect on their rate of decline [47]. However, DFO also binds aluminum, which has been suspected to play a role in the development of the disorder. Some chelators were reported for their ability to decrease the iron content of brain in a model of iron-loaded animals [48]. DFO and hydroxypyridinones (30-100 mg/kg) were found to significantly reduce the brain iron content; however, they

also induced a decrease in the level of dopamine, suggesting a removal of iron from tyrosine hydroxylase and therefore an inherent toxicity.

CURRENT IRON CHELATION STRATEGIES AND DRAWBACKS

Constraints which a clinically useful iron chelator must satisfy include high affinity for iron, specificity versus other metal ions, appropriate tissue distribution, metabolism and oral bioavailability (see references [5] and [8] for review). Careful attention should also be paid to redistribution of iron to more susceptible sites of the body and risk of encouragement of bacterial growth. Some of the powerful ferric iron chelators reported so far for treatment of systemic iron overload manage those constraints. However, few new chelating drug candidates have been dedicated to the treatment of oxidative stress associated diseases.

The potential use of siderophores and analogues for the treatment of systemic iron overload was recognised early [50, 51]. Siderophores have been evolved by microorganisms in order to provide iron for their growth. Most siderophores utilize three secondary hydroxamate or catecholate groups to provide very stable and specific octahedral coordination of ferric iron. Their affinity constants are extremely high, up to 10^{52} [7]. However, these agents, with the exception of DFO, suffer limitations for use *in vivo* due to their restricted bioavailability and propensity to remove ferric iron from both ferritin and transferrin. Another drawback for clinical use of natural siderophores is that they can deliver iron to some bacteria and fungi, thereby enhancing their pathogenicity. DFO has a low general acute toxicity [49]. However, severe toxic effects on visual, hearing and renal functions have been reported. Moreover, therapy with DFO has been associated with increased susceptibility to acute infections, thrombocytopenia and pulmonary complications. DFO also inhibits cell proliferation presumably by inhibiting ribunucleotide reductase [52]. Therefore, DFO can hardly be used for a long term treatment against oxidative damage associated conditions.

Hydroxypyridinone bidentate ligands have been widely investigated for ferric iron chelation. Their success is mainly due to their relative selectivity for ferric iron and to their unique ability to be uncharged under physiological conditions both as free ligand and as iron complex [49]. As a result, most of those compounds are orally active and cross cell membranes. However, their lipophilicity also enables them to penetrate numerous cells where they can exert undesired toxic effects. L1 was shown to have deleterious effects on bone marrow and to promote agranulocytosis during clinical studies. L1 also inhibits various iron containing enzymes, including tyrosine hydroxylase [53]. Although second generation hydroxypyridinone derivatives [54, 55] are now developed with a more favourable safety margin, it is unlikely that such compounds could be safely used against oxidative damage except in acute treatment. A new tridentate triazolyl iron chelator shown in (Fig. **3**) was very recently proposed as an alternative for L1 with an overall stability constant for ferric iron complexation of $10^{38.6}$ [56]. Pyridoxal isonicotinoyl hydrazones series [57] such as PIH (Fig. **3**) have also been widely investigated as bioavailable iron chelators. However, it must be outlined that bidentate or tridentate ligand can eventually form 1:1 iron chelates i.e. having free coordination sites, which therefore may be able to catalyse hydroxyl radical formation (*vide infra*). In contrast, HBED [58] and related compounds fully occupy the six coordination sites of iron (see Fig. **2**). HBED very efficiently inhibits the formation of hydroxyl radicals catalysed by iron, because it dramatically decreases its redox potential [59]. However, although these compounds and related prodrugs [60] look promising for the treatment of iron overload [61], their very high affinities for iron (10^{39} in the case of HBED) preclude their use for long term treatment in conditions unrelated to systemic iron overload.

ADDITIONAL CHEMICAL CONSTRAINTS FOR USE IN OXIDATIVE STRESS CONDITIONS

With a view to provide safe protection against oxidative damage, two issues have to be especially emphasized: (i) risk of iron depletion, i.e. risk of mobilizing essential iron from iron containing proteins and (ii) catalysis of Fenton chemistry.

Risk of Iron Depletion

Iron deficiency may result in anemia, but there is increasing evidence that it may also affect work performance, neurological function, immune response and epithelial tissues (see [11] and reference cited therein). Most powerful iron chelators are capable of removing ferric iron from iron containing proteins such as transferrin and from essential iron-containing enzymes, such as ribonucleotide reductase, tyrosine hydroxylase and lipoxygenases. These enzymes can be inhibited by: (i) removal of metal from active sites, (ii) formation of a ternary complex, (iii) depriving the apoenzyme of its normal source of iron. Moreover, although iron from hemoglobin is not readily removed, strong iron chelators may also interfere with the processing of iron incorporation into haem prosthetic group. From thermodynamic considerations, the higher is the stability constant for iron, the higher is the capacity of the chelator to remove iron from metalloproteins. In particular, if the affinity constant of the chelator for ferric iron is higher than the affinity constant of iron for transferrin, i.e. 10^{21}, the chelator is thermodynamically able to compete for iron bounded to this protein, and therefore iron depletion can be anticipated as a side-effect. Similarly, to compete for iron traces released during oxidative stress the association constant must be high enough as compared to those of endogenous nonspecific metal-binding molecules. Indeed, intracellular medium is rich in various small molecules able to nonspecifically bind iron, e.g. amino acids, nucleotides or citrate. It is noteworthy that the stability constant of ferric iron for citrate is $K_1=10^{11.4}$ [62]. The ability of a chelator to remove iron from iron proteins such as transferrin is not only based on thermodynamics but also on kinetic considerations. Rates of iron removal from transferrin may vary dramatically according to the chelator [63]. Finally, iron mobilization from iron containing proteins obviously depends on tissue distribution and especially on membrane permeability of the

chelator. Very hydrophilic iron chelators are likely to have only access to extracellular iron. However, some hydrophilic iron chelators might also access to cytosol by using specific transport systems. Conversely, relatively lipophilic chelators are more likely to have access to intracellular low molecular mass iron pool but also to intracellular iron containing proteins such as IRPs, with more potential side effects [64].

Catalysis of Fenton Chemistry

The ability of an iron chelate to catalyse Fenton chemistry is influenced by several thermodynamical and kinetic factors. The spin state of an iron complex conditions its possible interaction with oxygen and therefore its capacity to relieve the spin restriction for autoxidation of various ground state molecules. Fe(II) complexes can be high or low spin, depending on the ligand field of the coordinating molecule. In addition, the capacity of an iron chelate to catalyse Fenton chemistry is related to its redox potential. Generally, chelators in which oxygen atoms ligate the metal will tend to prefer the oxidized form. Thus, they decrease redox potential of Fe(II)/Fe(III). Conversely, chelators in which nitrogen atoms primarily bind the metal prefer ferrous form and tend to increase redox potential of iron [2]. To be able to catalyse the formation of hydroxyl radicals in the presence of hydrogen peroxide, an iron complex has first to fit with two simultaneous conditions [65]: (i) the ferric chelate has to be reducible by physiological reductants, i.e. its standard redox potential must be higher than -0.324 V / NHE ($NADPH/NADP^+$ redox couple), (ii) single electron transfer from ferrous chelate to hydrogen peroxide must be possible i.e. its redox potential must lie below +0.46 V / NHE (H_2O_2 / $HO^{\cdot}$, OH^-).

Iron-catalysed hydroxyl radical formation also requires at least one iron coordination site occupied by water or a readily dissociable group allowing access to superoxide and/or hydrogen peroxide [66]. This explains the ability of certain well-known iron chelates to be powerful catalysts of Fenton chemistry as shown in (Fig. **4**). Indeed, although EDTA is a potential hexadentate ligand, it is too small to reach all octahedric coordination sites. The structure of the ferric iron EDTA complex includes a seventh coordination site occupied by water [67]. Moreover, the redox potential of iron EDTA complex is E'° = +0.12 V / NHE, which means that it can alternatively be reduced by physiological reductants and oxidized by hydrogen peroxide, thus generating highly oxidizing species. On the other hand, the tri phenanthroline iron-complex (E'° = +1.15 V / NHE) does not react with hydrogen peroxide and the desferrioxamine-iron complex (E'° = -0.40V / NHE) cannot be reduced by physiological reductants [65]. However, such thermodynamical considerations refer to standard conditions at equilibrium, and it is likely that some reductases might be able to reduce iron even from ferrisiderophores such as ferrioxamine by continuously displacing the equilibrium in the presence of ferrous iron acceptors. Such a mechanism is probably involved in iron mobilization by various microorganisms. Some iron chelates may also be able to generate hydroxyl radicals in such a way that damage is directed to the chelator, thereby sparing more important targets which bind iron, such as DNA.

OXIDATIVE STRESS ACTIVATABLE IRON CHELATORS

Powerful chelators might be useful in acute situations e.g. for short periods of treatment during which temporary iron

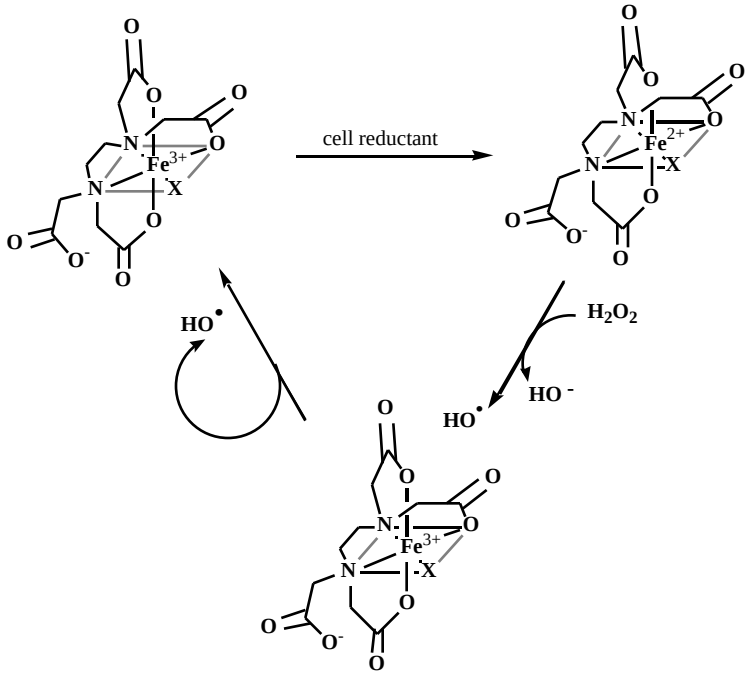

Fig. (4). Redox cycling of iron EDTA complex (X=unoccupied or readily exchangeable coordination site)

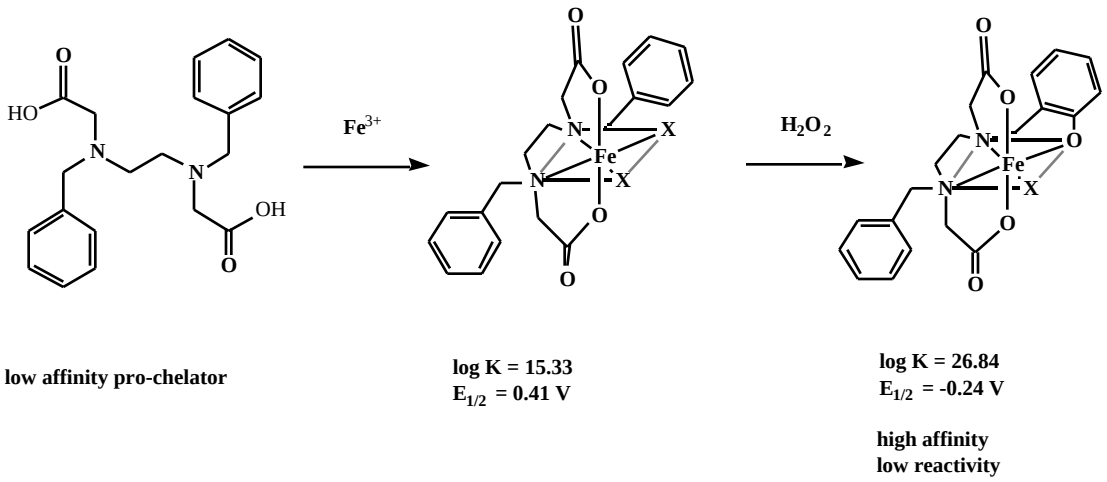

Fig. (5). Schematic oxidative activation pathway of the OR10141 series

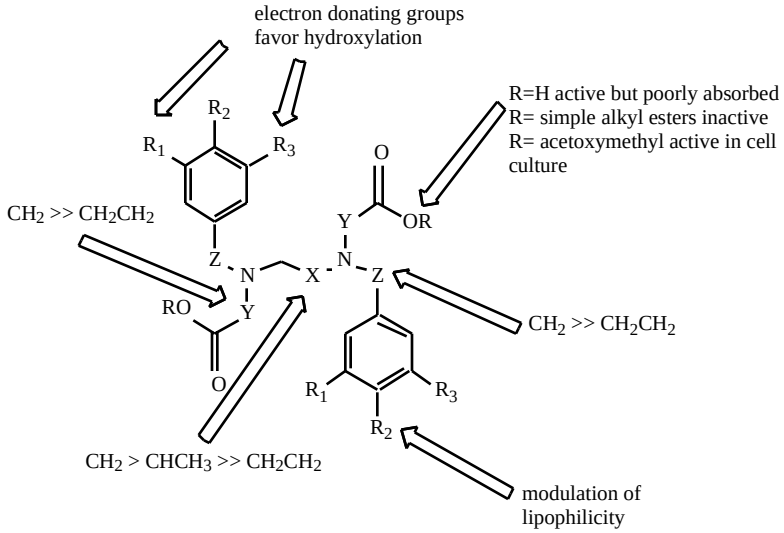

Fig. (6). Summary of structure activity relationships in the OR10141 series

deprivation and increase in iron excretion could be acceptable. Ischemia reperfusion injury or redox cycling xenobiotics poisoning treatment for instance may be managed by strong iron chelators. On the other hand, powerful iron chelators are not well suited for long-term treatment of oxidative stress-associated conditions such as chronic inflammatory disorders, atherosclerosis and possibly certain neurodegenerative diseases.

In order to overcome these problems, we recently developed a new strategy based on the idea that an « ideal » iron chelator to be used in oxidative stress-related conditions should not affect iron homeostasis, but should effectively chelate iron in an oxidative cellular environment. We explored the possibility of generating pro-drugs which can be directly activated by reactive oxygen species into species with strong iron binding capacity [68]. Of particular interest in such a strategy is the extent of the control exercised by oxidative stress conditions, whereby a dormant relatively inactive compound is transformed into an active species by the very conditions prevailing where it is most needed.

Substituted N,N'-dibenzyl ethylenediamine N,N'-diacetic acid derivatives were extensively studied as a paradigm for this new strategy. Compounds of this series have a low affinity for iron but undergo a site specific oxidation by hydrogen peroxide whereby they are converted into strong phenolate chelators related to HBED, which don't catalyse Fenton chemistry, as illustrated in (Fig. **5**). They have been designed to have an affinity for iron low enough ($K \# 10^{15}$) to avoid iron mobilization from iron proteins albeit able to bind free or loosely bound iron, i.e. iron intracellularly released by oxidative stress conditions. Intramolecular hydroxylation results in a dramatic 10^{11} increase in affinity constant for ferric iron, which is consistent with coordination by a hard phenolate donor group. Moreover, this dramatic increase in affinity for iron is associated with a large decrease in redox potential of Fe(II)/Fe(III) which tends to inhibit iron

reduction by physiological reductants thereby avoiding redox cycling of iron [69].

Intramolecular hydroxylation mechanisms as well as structure efficiency relationships were fully investigated in the series [70] and are summarized in (Fig. **6**). It was found that intramolecular aromatic hydroxylation is dependent on the chelating moiety and on the substituents of aromatic rings. The best profile was found with electron-enriched benzyl groups and ethylenediamine N,N'-diacetic acid or ethylenediamine N,N,N'-triacetic acid as chelating moiety, such as N,N'-Bis(3,4,5-trimethoxybenzyl)ethylenediamine N,N'-diacetic acid (OR10141). The X-ray structure of OR10141 iron complex has been determined, as well as insights towards the mechanisms of hydroxylation [71].

While it has a favorable preliminary toxicological profile [72], OR10141 has been shown to efficiently protect biological targets against oxidative damage in cell-free *in vitro* models. At 50-100 µM, it inhibits iron ascorbate-induced lipid peroxidation of rat liver microsomes and DNA single strand breaks, decreases the generation of carbonyl residues on various proteins and protects glucose 6-phosphate dehydrogenase against oxidative inactivation [73]. It suggests that the affinity of OR10141 for iron is high enough to partially withdraw non-specifically bound iron from target molecules in the above conditions. However OR10141 hardly protects intact cells against hydroperoxide toxicity except at high concentrations (i.e. >1 mM), because of poor membrane permeability. On the other hand, cytoprotective effects of various prodrugs or analogues with enhanced lipophilicity, to achieve higher intracellular levels of chelator, have been obtained using human skin fibroblasts. For instance, OR10141 acetoxymethyl ester efficiently protects cells in the low micromolar range [74], suggesting that, providing sufficient bioavailability is reached, OR10141 can afford an efficient protection against hydrogen peroxide, which also demonstrates the relevance of the oxidative activation pathway depicted in (Fig. **5**). Structural modifications of the core structure of OR10141 may further improve its bioavailability and enhance protective potency against oxidative injury while not interfering with iron homeostasis. It is noteworthy that carboxylate chelating moieties of OR10141 may be replaced by other monodentate coordinating ligands, such as aromatic nitrogen, to improve bioavailability, while still leading to intramolecular hydroxylation in certain conditions [75].

CONCLUDING REMARKS

Although a considerable work has been performed on iron chelation therapy against systemic iron overload, the treatment by iron chelators of pathological conditions associated with oxidative stress have been much less investigated. There is a need both for new drug candidates with good safety margins and for clinical studies to evaluate the usefulness of iron chelators in various oxidative stress-associated pathological conditions unrelated to systemic iron overload. DFO and hydroxypyridinones such as L1 are still good candidates for future investigation of acute oxidative stress situations, particularly because they can be used in humans. However, they also meet to serious limits due to their side effects when used for prolonged periods of time. The potential usefulness of an iron chelator which could be safely used in humans may be extremely broad. The « oxidative stress-activation » concept described in the present paper could provide a rational approach to minimize side effects and help developping iron chelating treatment against oxidative damage.

REFERENCES

[1] Halliwell, B.; Gutteridge, J.M.C. *Free Radicals in Biology and Medecine*, Clarendon, Oxford, **1989**.

[2] Miller, D.M.; Buettner G.R.; Aust S.D. *Free Rad. Biol. Med.* **1990**, *8*, 95-108.

[3] Halliwell, B. *J. Neurochem.* **1992**, *59*, 1609.

[4] Stadtman, E. R. *Free Rad. Biol. Med.* **1990**, *9*, 315.

[5] Hider, R. C.; Singh, S. *Procs. Roy. Soc. Ed.* **1992**, *99B*, 137.

[6] Nathan, D.G. *New Engl. J. Med.* **1995**, *332*, 953.

[7] Martell, A.E.; Anderson, W.F.; Badman, D. G. *Development of iron chelators for clinical use*, Elsevier North Holland, New York, **1981**.

[8] Porter, J.B.; Huens, E.R.; Hider R. C. *Bailliere's Clin. Haematol.* **1989**, *2*, 257.

[9] Ryan, T.P.; Aust, S. D. *Crit. Rev. Toxicol.* **1992**, *22*, 119.

[10] Reif, D.W. *Free Rad. Biol. Med.* **1992**, *12*, 417.

[11] Crichton R.R, *Inorganic Biochemistry of Iron Metabolism*, Elis Horwood, New York, London, **1991**.

[12] Fontecave, M.; Pierre, J.L. *Biochimie* **1993**, *75*, 767.

[13] Klausner, R.D.; Rouault, T.A.; Harford, J. B. *Cell* **1993**, *72*, 19.

[14] Watt, G.D.; Frankel R.B.; Papaefthymiou G. C. *Proc. Natl. Acad. Sci. USA* **1985**, *82*, 3640.

[15] Thomas, C.G.; Aust, S.D. *J. Biol. Chem.* **1986**, *261*, 13064.

[16] Jacobs, D.J.; Watt, G.D.; Frankel, R.B.; Papaefthymiou G. C. *Biochemistry* **1989**, *28*, 1650.

[17] Mazur A.; Baez, S.; Schorr, E. *J. Biol. Chem.* **1955**, *213*, 147.

[18] Biemond, P.; Swaak, A. J. G.; Beindorf, C. M.; Koster, J. F. *Biochem. J.* **1986**, *239*, 169.

[19] Pourzand, D.; Watkin, R.D.; Brown, J.E.; Tyrrell, R.M. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 6751.

[20] Fridovich I. *Arch. Biochem. Biophys.* **1986**, *274*, 1.

[21] Gardner, P.R.; Raineri, I.; Epstein, L.B.; White, C.W. *J. Biol. Chem.* **1995**, *270*, 13399.

[22] Liochev, S.I. *In Metal Ions in Biological Systems*, Marcel Dekker, New York, **1999**; Vol. 36, pp. 1-39.

[23] Atamna, H.; Ginsburg, H. *J. Biol. Chem.* **1995**, *270*, 24876.

[24] Trenam, C.W.; Blake D.R.; Morris, C.J. *J. Invest. Dermatol.* **1992**, *99*, 675.

[25] Sakaida, S.; Kyle, M.E.; Farber, J.L. *Mol. Pharmacol.* **1990**, *37*, 435.

[26] Blake, R.R.; Hall, N.D.; Bacon, P.A.; Dieppe, P.A.; Halliwell, B.; Gutteridge, J.M.C. *Ann. Rheum. Dis.* **1983**, *42*, 89.

[27] Polson, R.J.; Jawed, A.; Bomford, A.; Berry, H.; Williams, R. *Br. Med. J. (Clin. Res. Ed.)* **1985,** *291*, 448.

[28] Voest, E.E.; Vreugdenhil, G.; Marx, J.J.M. *Ann. Intern. Med.* **1994**, *120*, 490.

[29] Voogt, A.; Sluiter, W.; van Eijk H.G.; Koster, J.F. *J. Clin. Invest.* **1992**, *90*, 2050.

[30] Reddy, B.R.; Kloner, R.A.; Przyklenk, K. *Free Rad. Biol. Med.* **1989**, *7*, 45.

[31] Komara, J.S.; Nayini, N.R.; Bialik, H.A.; Indrien, R.J.; Evans, A.T.; Garritano, A.M.; Hoehner, T.J.; Jacobs, W.A.; Huang, R.R.; Krause, G.S.; White, B.C.; Aust, S.D. *Ann. Emerg. Med.* **1986**, *15*, 384.

[32] Van der Kraaij, A.M.M.; Van Eijk, H.G.; Koster, J.F. *Circulation* **1989**, *80*, 158.

[33] Herschko, C. *Mol. Aspects Med.* **1992**, *13*, 113.

[34] Braughler, J.M.; Hall, E.D.; Jacobsen, E.J.; McCall, J.M.; Means, E.D. *Drugs Future* **1989**, *14*, 143.

[35] Braughler, J.M.; Pregenzer, J.F.; Chase, R.L.; Duncan, L.A.; Jacobsen, E.J.; McCall, J.M. *J. Biol. Chem.* **1987**, *262*, 10438.

[36] Ryan, T.P.; Petry, T. W. *Arch. Biochem. Biophys.* **1993**, *300*, 699.

[37] Weiss, G.; Loyevsky, M.; Gordeuk, V.R. *Gen. Pharmacol.* **1998**, *32*, 155.

[38] Thomas, C.; Vile, G.F.; Winterbourn, C.C. *Biochem. Pharmacol.* **1993**, *45*, 1967.

[39] Malisza, K.L.; Hasinoff, B.B. *Arch. Biochem. Biophys.* **1995**, *316*, 680.

[40] Smith C.; Mitchinson, M.J.; Aruoma, O.I.; Halliwell, B. *Biochem. J.* **1992**, *286*, 901.

[41] Olszewer, E.; Sabbag, F.C.; Carter, J.P. *J. Natl. Med. Assoc.* **1990**, *82*, 173.

[42] Double, K.L.; Maywald, M.; Schmittel, M.; Riederer, P., Gerlach, M. *J. Neurochem.* **1998**, *70*, 2492.

[43] Gassen, M.; Youdim, M.B.H. *Pharmacol. Toxicol.* **1997**, *80*, 159.

[44] Grunblatt, E.; Mandel, S., Youdim, M.B.H. *Ann.N.Y. Acad Sci.* **2000**, *899*, 272.

[45] Sayre, L.M.; Perry, G.; Atwood, C.S.; Smith, M.A. *Cell. Mol. Biol.* **2000**, *46*, 731.

[46] Gerlach, M.; Ben-Shachar, D.; Riederer, P.; Youdim, M.B.H. *J. Neurochem.* **1994**, *63*, 793.

[47] McLachlan, D.R.C.; Dalton, A.J.; Kruck, T.P.A.; Bell, M.Y.; Smith, W.L.; Kallow, W.; Andrews, D.F. *Lancet* **1991**, *337*, 1304.

[48] Ward, R.J.; Dexter, D.; Florence, A.; Aouad, F.; Hiedr, R.; Jenner, R.; Crichton, R.R. *Biochem. Pharmacol.* **1995**, *49*, 1821.

[49] Porter J.B.; Huens E.R. *Bailliere's Clin. Haematol.* **1989**, *2*, 459.

[50] Bergeron, R.J.; Wiegand, J.; Dionis, J.B.; Egli-Karmakka, M.; Frei, J.; Huxley-Tencer; A.; Peter; H.H. *J. Med. Chem.* **1991**, *34*, 2072.

[51] Miller, M. J. *Chem. Rev.* **1989**, *89*, 1563.

[52] Bomford, A.; Isaac, J.; Roberts, S.; Edwards, A.; Young, S.; Williams, R. *Biochem. J.* **1986**, *236*, 243.

[53] Waldmeier, P.C.; Buchle, A.M.; Steulet, A.F. *Biochem. Pharmacol.* **1993**, *45*, 2417.

[54] Liu, Z.D.; Khodr, H.H.; Liu, D.Y.; Lu, S.L.; Hider, R.C. *J. Med. Chem.* **1999**, *42*, 4814.

[55] Rai, B.L.; Dekhordi, L.S.; Khodr, H.; Jin, Y.; Liu, Z.; Hider, R.C. *J. Med. Chem.* **1998**, *41*, 3347.

[56] Heinz, U.; Hegetschweiler, K.; Acklin, P.; Faller, B.; Lattmann, R.; Schnebli, H.P. *Angew. Chem. Int. Ed.* **1999**, *38*, 2567.

[57] Richardson, D.R. *Exp. Opin. Invest. Drugs* **1999,** *8*, 2141.

[58] L'Eplattenier, F.; Murase, I.; Martell, A.E. *J. Am. Chem. Soc.* **1967**, *89*, 837.

[59] Dean, R.T.; Nicholson, P. *Free Rad. Res.* **1994**, *20*, 83.

[60] Faller, B.; Spanka, C.; Sergejew, T.; Tschinke, V. *J. Med. Chem.* **2000**, *43*, 1467.

[61] Grady, R.W.; Hershko, C. *Ann. N. Y. Acad. Sci.* **1990**, *612*, 361.

[62] Singh S.; Hider, R.C. *Anal. Biochem.* **1988**, *171*, 47.

[63] Kretchmar Nguyen, S.A.; Craig A.; Raymond, K.N. *J. Am. Chem. Soc.* **1993**, *115*, 6758.

[64] Weiss, G.; Kastner, S.; Brock, J.; Thaler, J.; Grunewald, K. *Biochem. Pharmacol.* **1997**, *53*, 1419.

[65] Fontecave, M.; Pierre, J.L. *Bull. Soc. Chim. Fr.* **1993**, *130*, 77.

[66] Graf, E.; Mahoney, J.R.; Bryant, R.; Eaton, J.W. *J. Biol. Chem.* **1984**, *259*, 3620.

[67] Lind, M.D.; Hamor, M.J.; Hamor, T.A.; Hoard, J.L. *Inorg. Chem.* **1964**, *3*, 34.

[68] Galey, J.-B.; Dumats, J.; Beck, I.; Fernandez, B.; Hocquaux, M. *Free Rad. Res.* **1995**, *22*, 67.

[69] Serratrice, G.; Galey, J.-B.; Saint Aman, E.; Dumats, J. *Eur. J. Inorg. Chem.* **2001**, 471.

[70] Galey, J.-B.; Destrée, O.; Dumats, J.; Pichaud, P.; Marché, J.: Génard, S.; Bracciolli, G.; Le Capitaine, L.; Plessix,

H.; Brambilla, L.; Cantoni, O. *Free Rad. Biol. Med*. **1998**, *25*, 881.

[71] Ménage, S.; Galey, J.-B.; Dumats, J.; Hussler, G.; Séité, M.; Gauthier Luneau, I.; Chottard, G.; Fontecave, M. *J. Am. Chem. Soc*. **1998**, *120*, 13370.

[72] both acute and sub-chronical classical toxicological studies have shown no signs of toxicity up to 1 g OR10141 /kg/day (oral route). The compound is mainly excreted in urine and has a short plasma half life.

[73] Galey, J.-B.; Dumats, J.; Génard, S.; Destrée, O.; Pichaud, P.; Catroux, P.; Marrot, L.; Beck, I.; Fernandez, B.; Barre, G.; Seite, M.; Hussler, G.; Hocquaux, M. *Biochem. Pharmacol*. **1996**, *51*, 103.

[74] Galey, J.-B.; Destrée, O.; Dumats, J.; Génard, S.; Tachon, P. *J. Med. Chem*. **2000**, *43*, 1418.

[75] Mekmouch, Y.; Ménage, S.; Toia-Duboc, C.; Fontecave, M.; Galey, J.-B.; Lebrun, C.; Pécaut, J. *Angew. Chem. Int. Ed*. **2001**, *40*, 949.