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Manganese(III) Complexes of Bis-Hydroxyphenyldipyrromethenes are Potent Orally Active Peroxynitrite Scavengers

Smita Rausaria, **Andrew Kamadulski**, **Nigam P. Rath**[§], **Leesa Bryant**[†], **Zhoumou Chen**[†], **Daniela Salvemini**[†], and **William L. Neumann**[*]
Department of Pharmaceutical Sciences, School of Pharmacy, Southern Illinois University Edwardsville, Edwardsville, Illinois, 62026, Department of Pharmacology and Physiology, St. Louis University, St. Louis, Missouri, 63104, Department of Chemistry and Biochemistry and Center for Nanoscience, University of Missouri St. Louis, St. Louis Missouri, 63121

Abstract

We report a new series of bis-cyclohexano-fused Mn(III) complexes of bis-hydroxyphenyl-dipyrromethenes (DIPYs) **4a–c** as potent and orally active peroxynitrite scavengers. Complexes **4a–c** have been shown to reduce peroxynitrite through a 2-electron mechanism thereby forming the corresponding Mn(V)O species, which have been characterized by UV, NMR, and LCMS methods. Mn(III) complex **4b** and its strained BODIPY analogue **9b** have been analyzed by x-ray crystallography. Finally, complex **4a** has been shown to be an orally active and potent analgesic in a model carrageenan-induced hyperalgesia known to be driven by the overproduction of peroxynitrite.

The overproduction of reactive oxygen species (ROS) *in vivo* is now widely recognized as a key contributor to numerous pathologies.[1] One particularly damaging situation results from the diffusion controlled radical coupling of the central ROS, superoxide, with nitric oxide to form peroxynitrite.[2] The highly reactive peroxynitrite is a powerful biological oxidant which leaves a trail of dysfunctional oxidized and nitrated proteins, lipids and nucleotides, in its wake.[3] From a pharmacological perspective, peroxynitrite is considered a potent proinflammatory and proapoptotic species which plays a critical role in pain of several etiologies as demonstrated initially by our team and then by others.[4–6] Accordingly, the discovery of pharmaceutically relevant agents which can effectively decompose peroxynitrite should have significant therapeutic value.[2,3]

As a result of the early discoveries of Groves7 and Stern,[8] Mn(III) and Fe(III) porphyrins have emerged as an important class of peroxynitrite reductase and isomerase catalysts, respectively (Figure 1 A). Elegant mechanistic studies have revealed that the more pharmacologically-suitable Mn(III) porphyrins decompose peroxynitrite primarily in a one-electron fashion and require a biological co-reductant such as ascorbate to complete the reductase catalytic cycle.9 One electron reduction of peroxynitrite produces the potentially damaging nitrogen dioxide radical which is also thought to undergo rapid reduction by

[*] wneuman@siue.edu .
[§]University of Missouri St. Louis
[†]St. Louis University

Supporting Information Available: Experimental procedures, characterization data for all new compounds, LCMS, NMR, UV/Vis, boronate oxidation assay, oxidation studies, magnetic susceptibility measurement, pharmacological methods, and crystallographic data. This material is available free of charge via the internet at http://pubs.acs.org.

endogenous antioxidants.5 Thus, if endogenous antioxidants are plentiful, Mn(III)-pophryins can fully detoxify peroxynitrite *in vivo*. From this class, the isomeric Mn(III)-tetrakis-(*meso*-*N*-alkylpyridinium)porphyrins (e.g. Mn(III)-4-TMPyP^{5+}, **1**) are the most well-studied, both as peroxynitrite reductase catalysts and superoxide dismutase (SOD) mimics.[9,10]

Recently, Gross has reported that Mn(III) and Fe(III) corroles are also excellent peroxynitrite decomposition catalysts.[11] Remarkably, the Mn(III) corroles operate through a 2-electron cycle, reducing peroxynitrite to nitrite instead of nitrogen dioxide through a novel disproportionation mechanism. The most important finding from this work was that Mn(III) corroles can decompose peroxynitrite in a catalytic fashion (in contrast to Mn(III) porphyrins) and therefore do not require the assistance of endogenous co-reductants.

Although Mn(III) porphyrins, such as Mn(III)-4-TMPyP^{5+} **1**, and Mn(III) corrole systems, such as compound **2**, have proven to be powerful pharmacological tools in animal studies demonstrating the benefits of destroying peroxynitrite in vivo,[11−14] they are not optimal as therapeutic candidates. While these types of polycationic complexes have excellent catalytic activities and their high water solubility is a useful property for laboratory measurements, their corresponding high polarity renders them poorly membrane soluble. More amphiphilic metallocorroles have indeed shown great promise as orally active peroxynitrite decomposition catalysts.[12c] Unfortunately, synthetic methods for accessing polyfunctional corrole systems remain quite challenging and therefore not particularly suited for iterative structure-activity relationship (SAR) studies. To this end we have been keenly interested in the design, synthesis and evaluation of new catalyst systems with enhanced drug-like properties.

In our search for alternative trianionic ligand systems capable of supporting a Mn(V)O intermediate arising from the 2-electron reduction of peroxynitrite (similar to Mn(III) corroles), we were intrigued by the B,O-chelated boron-dipyrromethene (BODIPY®) dye **3** reported originally by Burgess (Figure 1 B).15 These dyes are constrained systems due to the chelate effect and thus have improved fluorescence properties over their non-chelated and well-known BODIPY congeners. The overall “ligand set” in these systems is not only perfect for mimicking the trianionic corrole but these compounds are also amenable to modular synthesis in good to excellent yields.[16] Herein we report the synthesis and evaluation of new bis-cyclohexano-fused Mn(III) complexes of bis-hydroxyphenyl-dipyrromethenes (DIPYs) as potent peroxynitrite scavengers with drug-like properties.

The syntheses commenced with readily available tetrahydroisoindole **5**[17] which was converted to the Boc-protected 3-bromo-tetrahydroisoindole **6** in 95% yield for two steps. Next, compound **6** underwent smooth Suzuki couplings with a representative set of protected hydroxyphenylboronic acids, **7a–c** in 70–89% yield, followed by a one pot decarboxylation/deprotection procedure to furnish the corresponding 2-benzyloxy- or 2-methoxyphenyl-tetrahydroisoindole derivatives **8a–c**. Compounds **8a–c** were then treated with benzaldehyde and TFA under Lindsey conditions[18] to form the corresponding dipyrromethane derivative, followed by oxidation to the dipyrromethene with p-chloranil[19] and subsequent phenol-ether deprotection with boron tribromide.[19,20] This sequence was carried out without intervening purifications. The boron tribromide deprotection step afforded the crude BODIPY systems **9a–c** which were then directly converted to the Mn(III) complexes **4a–c** by reaction with manganese(II) chloride under basic aerobic conditions. The dark emerald green complexes **4a–c** behave as simple lipophilic “organic” molecules and were thus amenable to purification by flash chromatography on silica gel providing analytical materials for characterization and activity studies. The overall yield of this sequence ranged from 16–32% (unoptimized) and is therefore quite competitive with

porphyrin and corrole syntheses which require the preparation of functionalized pyrrole and/or dipyrromethane units followed by an often low yield cyclization/oxidation step.[18]

Magnetic susceptibility measurement confirmed that representative complex **4b** is high-spin d^4 Mn(III). ^{1}H NMR spectra were recorded for **4a** in CD_2Cl_2 which revealed broad and shifted peaks indicative of the paramagnetic Mn(III) complex. Oxidation of **4a** with m-CPBA in CD_2Cl_2 afforded normal sharpened peaks characteristic of the corresponding diamagnetic low-spin d^2 Mn(V)O complex (Figures S21 and S22).[21]

Treatment of a green-colored methanolic solution of each Mn(III) complex **4a–c** with excess peroxynitrite (in 0.1N NaOH)[22] afforded the corresponding red-colored Mn(V)O intermediate (Figure 2). In methanol solution, the Mn(V)O species persist for 20–30 minutes and are stable enough to have been confirmed by LCMS (Figure 2 inset) and UV spectroscopy. The UV/Vis spectral changes are analogous to those observed during the oxidative generation and subsequent reduction of Mn(V)O-corroles[23] and in the elegant studies of highly functionalized Mn(V)O-corrolazines reported by Goldberg.21 In all three complexes (**4a–c**) the oxo-species are clearly observable using positive ion mode LCMS as $[M + H]^+$ species confirming the formal oxidation state of $[Mn(V)O]^{+3}$ (accounting for the trianionic ligand set). Treatment of the methanolic solutions of Mn(V)O species with 5 equivalents of ascorbate in phosphate buffer (pH = 7.2) resulted in the apparent instantaneous conversion back to the green Mn(III) form, effecting a possible reductase mode of action.

The results shown in Figure 2 are of interest only for identifying the putative 2-electron oxidation of **4b** with subsequent study of the Mn(V)O species **10b** by spectroscopic methods. However, to confirm that complexes can indeed decompose peroxynitrite more rapidly than its spontaneous decomposition at physiological pH, a relevant rapid throughput in vitro assay was sought.

Complexes **4a–c** were therefore assayed for their peroxynitrite decomposition activity by determining their ability to inhibit aryl boronate oxidation.[24] Oxidation of 4-acetylphenylboronic acid to 4-acetylphenol by peroxynitrite is a clean conversion devoid of any observable intermediates and the second order rate constant for this reaction has been accurately measured at $k = 1.6 \times 10^6\ M^{-1}s^{-1}$ by stopped-flow spectrophotometric methods.[24] Inhibition results for complexes **4a–c** and Mn(III)-4-TMPyP^{5+}, **1** are presented in Table 1. From the percent inhibition values we have calculated the corresponding apparent second order rate constants for oxidation of the Mn(III) form to the Mn(V)O form for **4a–c** and the Mn(IV)O form for **1** at 25 °C in phosphate buffer (pH = 7.2). The numbers match up well for the rate constants reported in the literature for the well known complex **1.**[25] Thus, this assay provides a reliable method for the rapid *in vitro* measurement of activity toward decomposing peroxynitrite prior to *in vivo* studies. Our data reveals that analogues **4a–c** have estimated apparent rate constants in the range of those observed for manganese corrole systems.[11] If the resulting Mn(V)O forms are more rapidly reduced back to the Mn(III) resting state by endogenous reductants, the k values in Table 1 are thus the catalytic rate constants for reductase-type activity.[9] In that manganese corroles have been shown to possess peroxynitrite decomposition activity without the need for endogenous reductants,[11] complexes **4a–c** may also operate via a similar catalytic cycle as they possess a similar trianionic ligand environment. Future mechanistic studies will address this possible mode of catalysis.

Single crystal x-ray analysis of **4b** and its BODIPY analogue and synthetic precursor **9b** provided very interesting structural information for this new ligand class (Figure 3). The

BODIPY system **9b** shows a significant distortion of the tetrahedral boron center, similar to that observed for the related non-cyclohexano system.[15]

In the case of **9b**, the O-B-O angle is 108.4, but the N1-B-O2 and N2-B-O1 angles are 114.4 and 113.1 respectively. The N-B-N angle is pinched in to 106.5 most likely in response to the phenyl bisecting the planar dipyrromethene unit and therefore feeling the close steric interaction of the CH_2 groups of the neighboring fused-cyclohexano rings. The Mn(III) complex **4b** crystallized with coordinated axial methanol molecules (from CH_2Cl_2-MeOH solution) to afford a Jahn-Teller distorted octahedral array around the manganese atom. The phenyl-dipyrromethene unit in **4b** is displaced upward relative to the equatorial plane with the coordinating phenolate groups displaced slightly downward. The release in strain associated with the distorted tetrahedron of **9b** most likely drives its direct conversion to **4b** without the need for isolation of the free phenol ligand. Both axial Mn-O(MeOH) bonds are considerably longer than in similar structures observed for Mn(III) corroles (2.19 Å)[11c] and Mn(III) corrolazines (2.107 Å)21b most likely due to the Jahn-Teller effect.26 The Mn-O3 bond distance is 2.226 Å and the longer Mn-O4 bond distance is 2.342 Å, possibly also influenced by steric interactions with the saddled hydroxyphenyl groups.

In the well known Hargreaves model[28] intraplantar injection of carrageenan led to the time-dependent development of thermal hyperalgesia in rats, an inflammatory response known to be driven by high levels of peroxynitrite flux (Figure 3).[29] This hyperalgesic effect was potently inhibited by **4a** when given by oral gavage (Figure 4). Nearly 100% inhibition is seen for 2 h with a substantial inhibitory effect maintained out to 5 h. At the 2h time point, a similar degree of inhibition was observed with the with the non-selective COX-1/COX-2 inhibitor ibuprofen at 300 mg/kg (99±5% inhibition, n=6). Under the same conditions and at 300 mg/kg, acetaminophen or aspirin attenuated hyperalgesia by 20±4% and 50±6% respectively (n=6, $P < 0.05$ compared to carrageenan alone at the 2h time point). Complexes **4b** and **4c** have also been shown to possess potent oral activity in this model. All three new complexes have LogP values in the range of +4, indicating high lipid solubility while Mn(III)-4-TMPyP^{5+} **1** and related catalysts possess highly negative values (Table 1).[30]

Both **4b** and **4c** were designed to divert or block the potential metabolic hydroxylation para to the chelated phenol through methyl- and fluoro-substitution, respectively. Full SAR studies incorporating further electron donating and withdrawing functionality will be the subject of future reports.

In conclusion, the results presented herein demonstrate the modular synthesis of a new class of orally active Mn(III) complexes which function as peroxynitrite scavengers. These studies suggest that this new complex design may afford improved in vivo performance through the 2-electron reduction of peroxynitrite to nitrite.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

(1). Batinic-Haberle I, Reboucas JS, Spasojevic I. Antioxid. Redox Signal. 2010; 13:877–918. [PubMed: 20095865]

(2). Ferrer-Sueta G, Radi R. ACS Chem. Biol. 2009; 4:161–177. [PubMed: 19267456]

(3). Szabo C, Ischiropoulos H, Radi R. Nat. Rev. Drug Discov. 2007; 6:662–80. [PubMed: 17667957]

(4). Salvemini D, Neumann W. Life Sci. 2010; 86:604–14. [PubMed: 19576230]

(5). Salvemini D, Neumann WL. Trends Pharmacol. Sci. 2009; 30:194–202. [PubMed: 19261337]

(6). Salvemini D. Arch. Biochem. Biophys. 2009; 484:238–44. [PubMed: 19017525]

(7). Groves JT, Marla SS. J. Am. Chem. Soc. 1995; 117:9578–9579.

(8). Stern MK, Jensen MP, Kramer K. J. Am. Chem. Soc. 1996; 118:8735–8736.

(9). Lee J, Hunt JA, Groves JT. J. Am. Chem. Soc. 1998; 120:6053–6061.

(10). Ferrer-Sueta G, Vitturi D, Batinic-Haberle I, Fridovich I, Goldstein S, Czapski G, Radi R. J. Biol. Chem. 2003; 278:27432–8. [PubMed: 12700236]

(11). (a) Mahammed A, Gross Z. Angew. Chem. Int. Ed. 2006; 45:6544–7. (b) Kupershmidt L, Okun Z, Amit T, Mandel S, Saltsman I, Mahammed A, Bar-Am O, Gross Z, Youdim MB. J. Neurochem. 2010; 113:363–73. [PubMed: 20096090] (c) Gershman Z, Goldberg I, Gross Z. Angew. Chem. Int. Ed. 2007; 46:4320.

(12). (a) Okun Z, Kupershmidt L, Amit T, Mandel S, Bar-Am O, Youdim MBH, Gross Z. ACS Chem. Biol. 2009; 4:910. [PubMed: 19715343] (b) Kanamori A, Catrinescu M-M, Mahammed A, Gross Z, Levin LA. J. Neurochem. 2010; 114:488–498. [PubMed: 20456018] (c) Haber A, Mahammaed A, Fuhrman B, Volkova N, Coleman R, Hayek T, Aviram M, Gross Z. Angew. Chem. Int. Ed. 2008; 47:7896.

(13). Drel VR, Pacher P, Vareniuk I, Pavlov I, Ilnytska O, Lyzogubov VV, Tibrewala J, Groves JT, Obrosova IG. Eur. J. Pharmacol. 2007; 569:48–58. [PubMed: 17644085]

(14). Vareniuk I, Pavlov IA, Drel VR, Lyzogubov VV, Ilnytska O, Bell SR, Tibrewala J, Groves JT, Obrosova IG. Exp. Neurol. 2007; 205:425–36. [PubMed: 17475250]

(15). Kim H, Burghart A, Welch MB, Reibenspies J, Burgess K. Chem Commun (Camb). 1999:1889–1890.

(16). Burghart A, Kim H, Welch MB, Thoresen LH, Reibenspies J, Burgess K. J. Org. Chem. 1999; 64:7813–7819.

(17). May J, D.A. Lash TD. J. Org. Chem. 1992; 57:4820–4828.

(18). (a) Lindsey JS, Schreiman IC, Hsu HC, Kearney PC, Marguerettaz AM. J. Org. Chem. 1987; 52:827. (b) Littler BJ, Ciringh Y, Lindsey JS. J. Org. Chem. 1999; 64:2864–2872. [PubMed: 11674358]

(19). Parhi AK, Kung MP, Ploessl K, Kung HF. Tetrahedron Lett. 2008; 49:3395–3399. [PubMed: 19122762]

(20). Loudet A, Bandichhor R, Burgess K, Palma A, McDonnell SO, Hall MJ, O'Shea DF. Org. Lett. 2008; 10:4771–4774. [PubMed: 18816131]

(21). Similar behavior was observed for the manganese(V)-oxo corrolazines: (a) Mandimutsira BS, Ramdhanie B, Todd RC, Wang H, Zareba AA, Czernuszewicz RS, Goldberg DP. J. Am. Chem. Soc. 2002; 124:15170. [PubMed: 12487581] . (b) Lansky DE, Mandimutsira BS, Ramdhanie B, Clausen M, Penner-Hahn J, Zvyagin SA, Telser J, Kryzstek J, Zhan R, Ou Z, Kadish KM, Zakharov L, Rheingold AL, Golberg DP. Inorg. Chem. 2005; 44:4485. [PubMed: 15962955] . (c) Goldberg DP. Acc. Chem. Res. 2007; 40:626. [PubMed: 17580977] .

(22). Uppu RM, Pryor WA. Anal. Biochem. 1996; 236:242–9.

(23). Liu HY, Yam F, Xie YT, Li XY, Chang CK. J. Am. Chem. Soc. 2009; 131:12890–1. [PubMed: 19737012]

(24). Sikora A, Zielonka J, Lopez M, Joseph J, Kalyanaraman B. Free Radic. Biol. Med. 2009; 47:1401–7. [PubMed: 19686842]

(25). Hunt JA, Lee J, Groves JT. Chem. Biol. 1997; 4:845–58. [PubMed: 9384531] (b) Differences in pH (7.2 versus 7.4) for the boronate assay versus stopped flow experiments are in part responsible for the minor differences in the kinetic data.

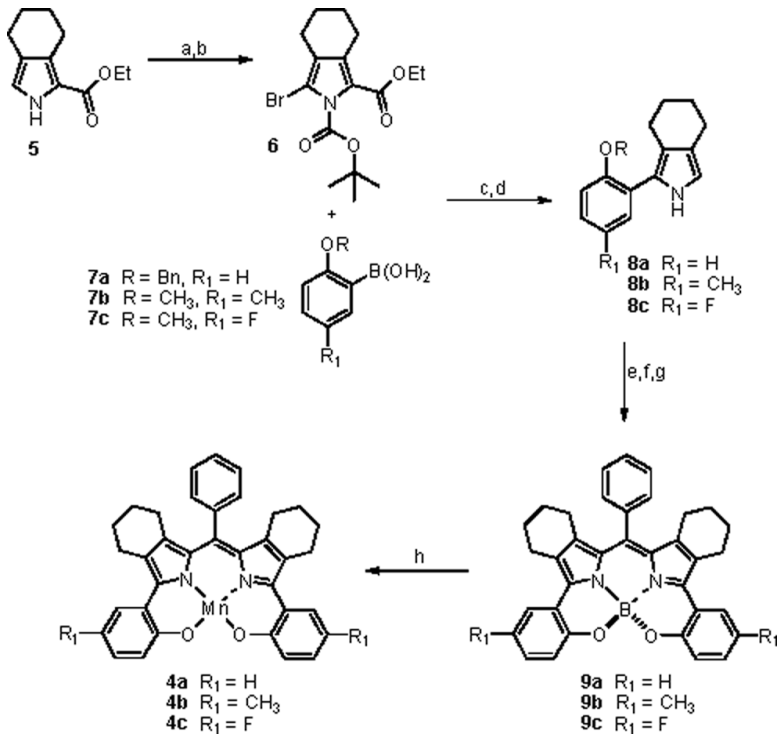
(26). (a) Lieb D, Zahl A, Shubina TE, Ivanovic-Burmazovic I. J. Am. Chem. Soc. 2010; 132:7282. [PubMed: 20462177] (b) Hoogenraad M, Ramkisoensing K, Driessan WL, Kooijman H, Spek AL, Brouwman E, Haasnoot JG, Reedijk J. Inorg. Chim. Acta. 2001; 320:117.

(27). Lodge K. J. Chem. Eng. Data. 1999; 4:1321–1324.

(28). Measurement of paw-withdrawl latency from a thermal stimulus (pawwithdrawl from a warm surface): Hargreaves K, Dubner R, Brown F, Flores C, Joris J. Pain. 1988; 32:77–88. [PubMed: 3340425] .

(29). Salvemini D, Wang ZQ, Bourdon DM, Stern MK, Currie MG, Manning PT. Eur. J. Pharmacol. 1996; 303:217–20. [PubMed: 8813572]

(30). (a) Kos I, Benov L, Spasojevic I, Reboucas JS, Batinic-Haberle I. J. Med. Chem. 2009; 52:7868–72. [PubMed: 19954250] (b) Batinic-Haberle I, Ndengele MM, Cuzzocrea S, Reboucas JS, Spasojevic I, Salvemini D. Free Radic. Biol. Med. 2009; 46:212–9. [PubMed: 18983908]



Scheme 1.

Synthesis of Mn(III) complexes of bishydroxyphenyl-DIPYs[a]

[a](a) NBS, THF, 100%, (b) Boc_2O, 4-DMAP, CH_3CN, 95% (c) 5% $Pd(PPh_3)_4$, Na_2CO_3-H_2O, CH_3OH, toluene, 70–89%, (d) $(CH_2OH)_2$, KOH, 195 °C, 70–85%, (e) PhCHO, cat. TFA, 52–61%, (f) *p*-chloranil, CH_2Cl_2, (g) BBr_3, CH_2Cl_2, (h) $MnCl_2$, $CHCl_3$, CH_3OH, 2,6-Lutidine, air, 68–79% for 3 steps (from f).

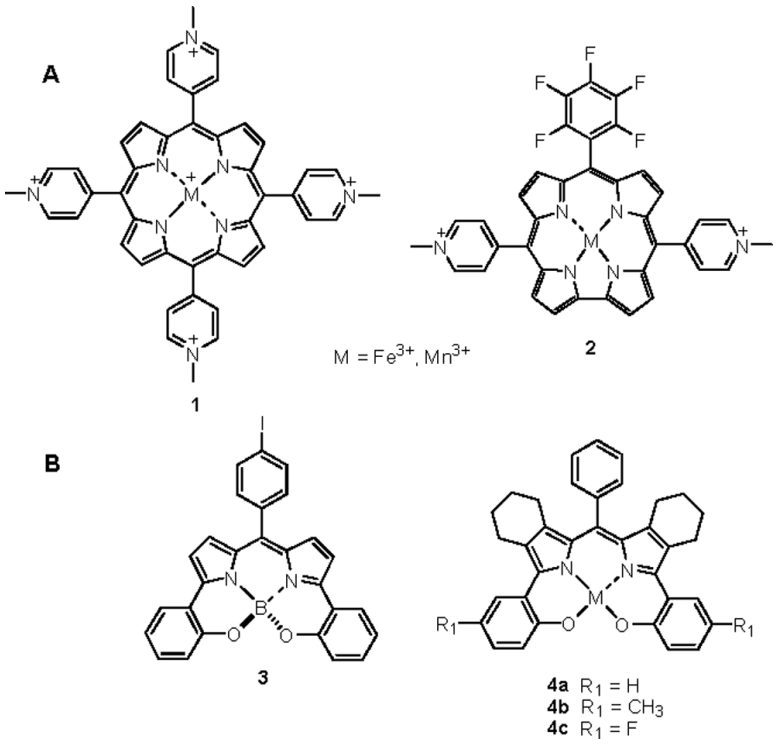


Figure 1.
(A) Peroxynitrite decomposition catalysts **1** and **2**. (B) Bishydroxyphenyldipyrromethene analogues **3** and **4**.

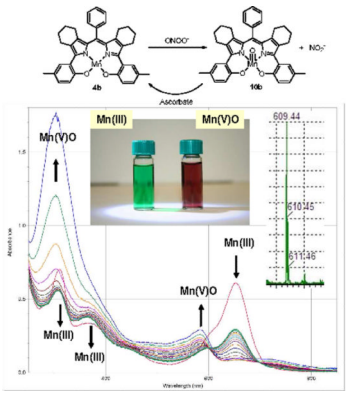


Figure 2.

UV-Vis spectral changes for **4b** (33 ηM, MeOH, 25 °C) after treatment with 0–20 equivalents peroxynitrite (spectra collected every 2 min. See supplementary data) Center inset: samples of **4b** and **10b**. Right inset: LCMS [M+H]+ for Mn(V)O specie **10b**.

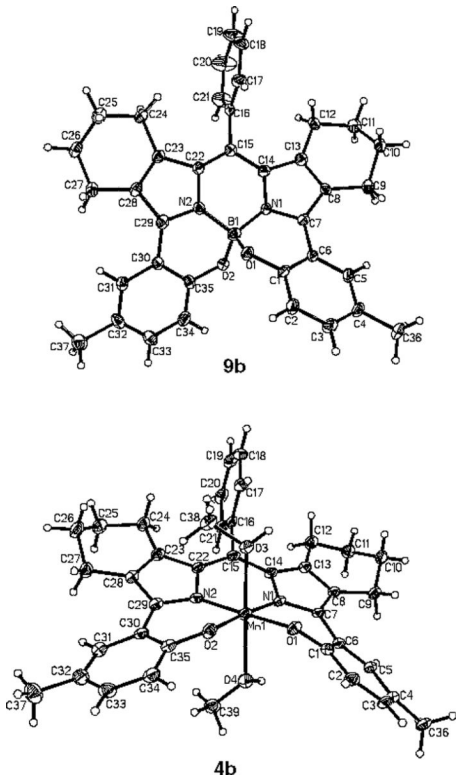


Figure 3.
Single crystal structural analysis for BODIPY analogue **9b** and manganese(III) complex **4b**.

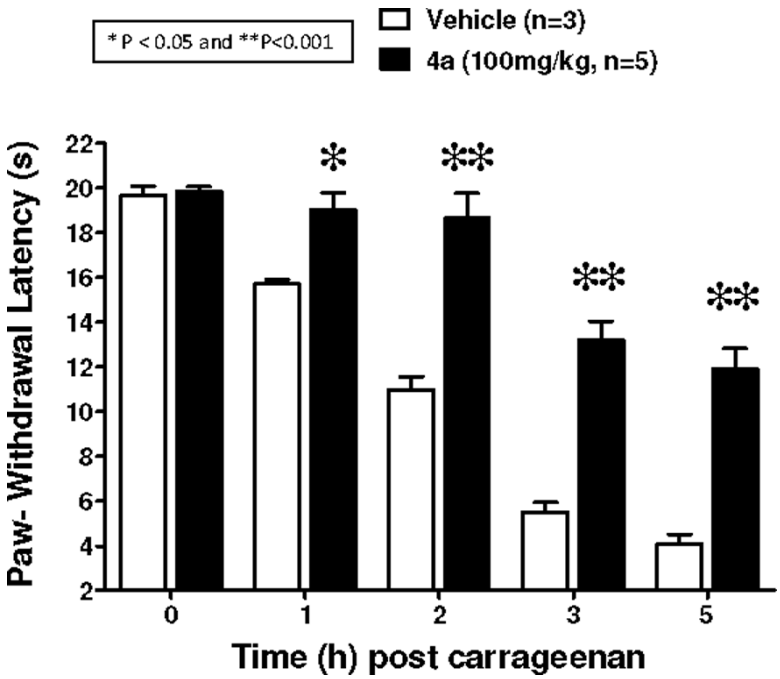
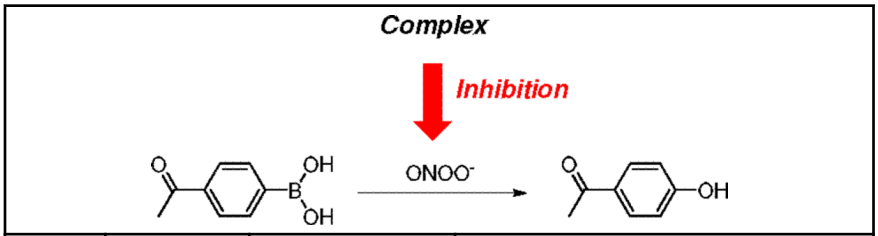


Figure 4.

The time-dependent development of carrageenan-induced thermal hyperalgesia (n=3) in rats was blocked by oral administration of **4a** (100 mg/kg, n=5). Results are expressed as mean ± SEM and analyzed by two-way ANOVA with Bonferroni *post hoc* tests where ***P*<0.05 and ***P*<0.001 *vs.* Vehicle (n = number of animals).

Table 1

Inhibition of Arylboronic Acid Oxidation



Catalyst	LogP[a]	% Inhibition (25 °C)	Second-order rate constant, k, $M^{-1}s^{-1}$ (25 °C)
4a	+4.18 ± 0.19	32.0 ± 2.40	[b] $7.5 \pm 0.6 \times 10^5$
4b	+4.31 ± 0.02	25.3 + 2.21	[b] $5.4 \pm 0.5 \times 10^5$
4c	+4.05 ± 0.15	31.5 ± 2.50	[b] $7.3 \pm 0.6 \times 10^5$
1	−4.54 ± 0.16	62.4 ± 0.68	[b] $2.6 \pm 0.2 \times 10^6$ [c] 1.8×10^6

[a] Measured by the slow-stir method[27]

[b] apparent second order rate constant for the oxidation of complex (1 equivalent) by peroxynitrite (1 equivalent) estimated from % inhibition in 100 mM phosphate buffer (pH = 7.2); no secondary antioxidants added; determined by LCMS after 1 min reaction time

[c] second order rate constant measured by stopped flow methods.[25]