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Synthesis of a Hybrid between SOD Mimetic and Ebselen to Target Oxidative Stress

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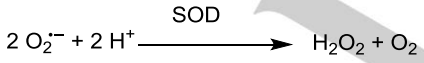
Abstract: Oxidative stress emerged as target in drug discovery due to its diverse role in various diseases, such as cardiovascular, neurodegenerative and autoimmune diseases. During the last decades, considerable progress was made in the development of compounds with the ability to reduce reactive oxygen species (ROS) like superoxide. However, the dismutation of the latter leads to formation of another harmful ROS, hydrogen peroxide, which can be depleted through peroxidase activity. The present work describes the synthesis of a hybrid, which unifies a superoxide dismutase mimetic (Mn(II)pyane) and a glutathione peroxidase mimetic (ebselen) that are connected via an amide bond. This unique hybrid is designed in order to convert superoxide into oxygen and water, i.e. as a potential biological agent for complete ROS removal, and will be used in the future as mechanistic molecular tool for further elucidation of (patho) physiological consequences of ROS removal.

The superoxide anion radical is considered to be a reactive oxygen species (ROS).[1] Although it naturally occurs in the body as a product of autoxidation or enzyme catalyzed oxidative processes, such as those driven by xanthine oxidase, lipoxygenase, cyclooxygenase or NADPH dependent oxidase, and it is involved in signaling pathways of synaptic plasticity and memory formation, an imbalance of superoxide is linked to aging processes, cancer, cardiovascular and neurodegenerative diseases.[1-2] The enzyme superoxide dismutase (SOD) should balance its steady-state concentration in living organisms by catalyzing superoxide decomposition to hydrogen peroxide and oxygen (Scheme 1).[3]



Scheme 1. Disproportion of superoxide to hydrogen peroxide and oxygen via superoxide dismutase (SOD).[3]

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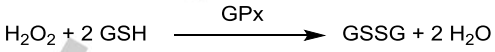
Hydrogen peroxide in general can damage biomolecules. It can impair the function of enzymes involved in energy metabolism and is capable of DNA damage by its reaction with transition metal ions or by radiation with UV light to form hydroxyl radical.[1, 4] Hence, the presence of peroxide cleaving enzymes like glutathione peroxidase (GPx) utilizing glutathione (GSH) as reductant is crucial for maintaining healthy ROS levels in the body (Scheme 2).[5]



Scheme 2. GPx catalyzed reaction of peroxide with glutathione GSH.[5]

The organoselenenium compound ebselen (Figure 1) has gained large interest, not merely due to its GPx mimicking abilities, but furthermore, since it is widely applicable as biological antioxidant in the treatment of neurodegeneration, bipolar disease, diabetes, cancer and due to its antibacterial, antimycotic and antiviral effects.[6] The only drawback is the sparse solubility in aqueous media causing difficulties in administration as a drug.[6e]
Among the superoxide dismutase mimetics (SODm), manganese(II) pentaazamacrocycles (MnPAMs) were observed to have remarkable SOD activity and beneficial biological effects.[7] Mn(II)pyane is a prototype of MnPAMs, which has been extensively studied in chemical settings as well as in cellular milieu and *in vivo*.[7e, 8] It has also been functionalized by us for the purposes of different studies.[8b, 8c, 9] One of these derivatives includes a primary amine residue suitable as a linker for hybridization with another moiety.[8b, 9a]

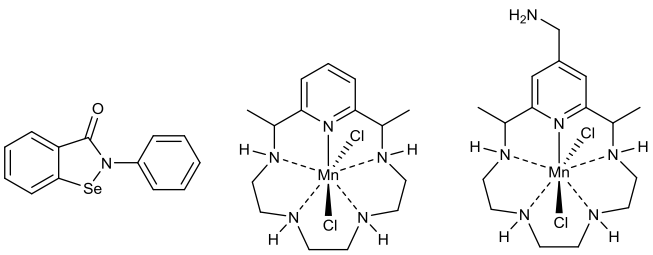


Figure 1. Structures of ebselen (left) Mn(II)pyane (middle) and Mn(II)pyane-CH_2NH_2 (right).[6a, 8b]

The combination of two or more active agents covalently bonded is long known as the concept of hybridization and represents a

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sophisticated approach aiming at several targets.[10] In the past, several examples have demonstrated the advantages of hybridization regarding improved biological activity with remaining minimal toxicity and potential to overcome drug resistances.[10-11] To eliminate oxidative stress and its accompanying damage, we designed a hybrid molecule of Mn(II)pyane-CH_2NH_2 and ebselen (Figure 2) to target both superoxide, with the former, and hydrogen peroxide, with the latter, potentially resulting in water and oxygen as products, i.e. complete ROS removal. Compounds with this synergistic mode of action include manganese salen complexes (EUK series), porphyrins, peptides and organoselenium-modified β-cyclodextrins to name a few and represent a promising class of ROS scavengers.[12]

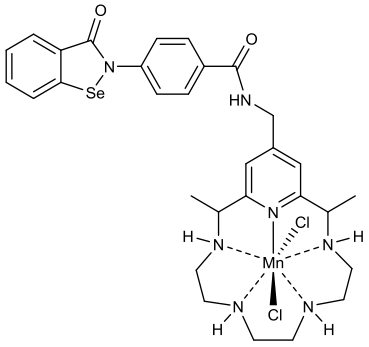

Figure 2. Newly synthesized Mn(II)pyane/ebselen hybrid (**6**).

All synthetic steps for the hybrid are described in Figure 3. Starting from benzocaine, which represents a cheap and available starting material, ethyl 4-(2-iodobenzamido)benzoate (**1**) is formed in reaction with the activated acid chloride 2-iodobenzoyl chloride in yields of 90 %.[13] The ring closure to form the isoselenazolone **2** was achieved in good yields by preparation of KSeO^{t}Bu with selenium powder and potassium tert-butoxide in dimethylformamide to react with **1**.[14] Quantitative hydrolysis of the ethyl ester was achieved with 5 equivalents of lithium hydroxide to yield ebselen acid (**3**) in a mixture of tetrahydrofuran, methanol and water.[15] To activate the carboxylic acid function, *N*-hydroxysuccinimide in combination with (1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCxHCl) was used and extraction with water facilitated the removal of the urea byproduct.[16] After purification by column chromatography the *N*-hydroxysuccinimide ester can finally be coupled to the amine of the Mn(II)pyane-CH_2NH_2 (**5**), which was synthesized by our group earlier.[8b] The formed Mn(II)pyane/ebselen hybrid **6** is extracted with water from the organic phase, which reveals that ebselen's insolubility in polar solvents is eradicated by coupling with the Mn(II)pyane moiety.[6e] All compounds were characterized by elemental analysis, UHR ESI-MS and ^{1}H and ^{13}C NMR, respectively. The spectra are included in the supporting information.

We have also studied the SOD and GPx activity of the hybrid. The obtained SOD activity, with a catalytic rate of k_{cat} = 1.35(1) x 10^7 $M^{-1}s^{-1}$ (MOPS buffer, pH 7.4), is - within the error limit- the same as the one for its mother-compound Mn(II)pyane (Figure S20). The GPx activity of the hybrid was determined using the reductase coupled assay, which resulted in a catalytic rate of k_{cat} = 0.022 ± 0.003 $M^{-1}s^{-1}$ (Figures S21). By way of comparison, for ebselen alone we obtained a catalytic rate of k_{cat} = 0.02 ± 0.01 $M^{-1}s^{-1}$ (Figure S22), being in accordance with the literature value (k_{cat} = 0.0216 $M^{-1}s^{-1}$).[17] Thus, as we could experimentally observe the SOD and GPx activity of the two bioactive units remains unchanged upon their chemical coupling. This is an important property required for the future studies, in order to correctly evaluate a possible differences between biological effects of the hybrid and its separate components (Mn(II)pyane and ebselen), as well as their mixtures.

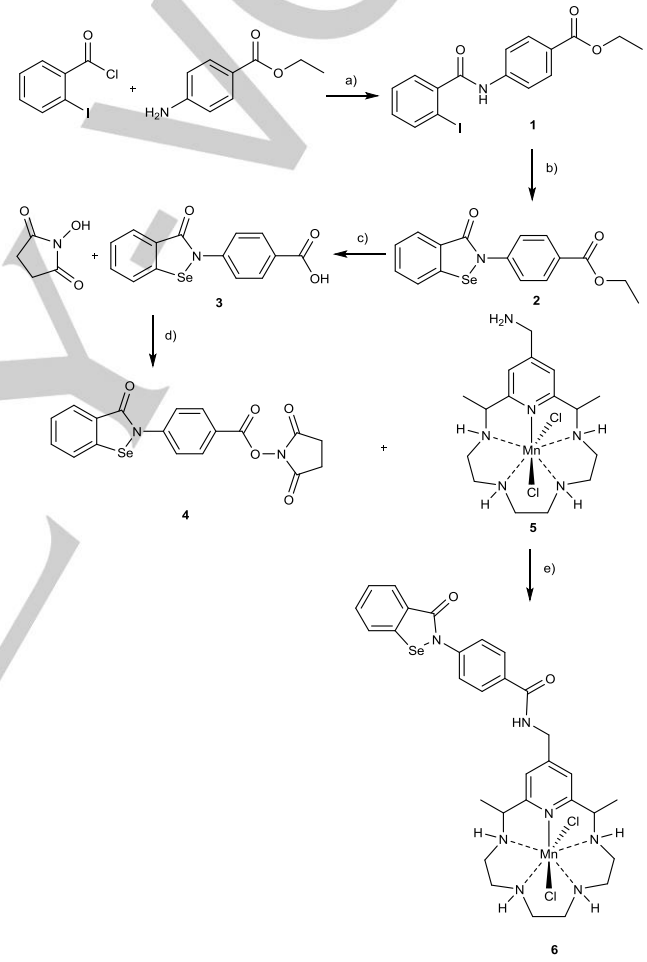

Figure 3. Synthesis of Mn(II)pyane/ebselen hybrid **6**. Reaction conditions: a) NEt_3 (1.0 eq) in DCM, RT, 24 h; [13] b) KO^{t}Bu (1.6 eq) and selenium powder (1.3 eq) in DMF, 60 °C, 48 h; [14] c) LiOH x H_2O (5.0 eq) in THF/MeOH/H_2O (2:1:1), RT, 4 days, acidic workup; [15] d) *N*-Hydroxysuccinimide (1.5 eq) and EDCxHCl (1.5 eq) in DCM/THF (1:1), 67 °C, 17 h, washed with water; [18] e) in DCM and MeOH (2:1), RT, 72 h, extraction with water.[8b, 16, 18]

In conclusion, we describe the synthesis of a Mn(II)pyane/ebselen hybrid with a potential synergic SOD and GPx activity combined in one molecule. The two separate functionalities Mn(II)pyane and ebselen were studied for years and are promising candidates in balancing ROS: the former catalyzes the conversion of superoxide to water and hydrogen peroxide, the latter, in combination with the cellular widely available tripeptide glutathione, is capable of cleaving the peroxide, protecting against cell damage. Solubility in water of the hybrid **6** is a major advantage regarding future *in vitro* and *in vivo* testing and is an enormous improvement when compared to ebselen. The molecule delivers new opportunities as a potential agent against two significant reactive oxygen species, superoxide and hydrogen peroxide and as such, it could be applied as mechanistic tool to further decipher the role of these two ROS entities in physiological and pathophysiological processes. Knowing that the applicability of both components ranges from activity against cancer, Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis (ALS), bipolar disorder, diabetes over the process of aging to antibacterial, antimycotic and antiviral effects, it is expected that the obtained hybrid will become an attractive object for diverse studies.[2b, 6] In future investigations, its activity will be compared with that of its pure components as well as with the activity of their mixture. In that way we will be able to judge whether a covalent coupling of two components brings advantages over their physical mixture.

Acknowledgements

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Keywords: SOD mimetic • GPx mimetic • ebselen • ROS • manganese complexes

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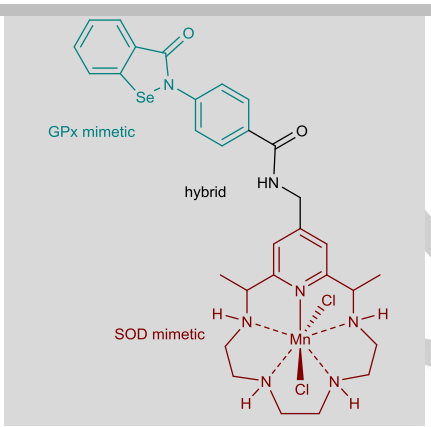
Suggestion for the Entry for the Table of Contents

COMMUNICATION

Newly synthesized tool against oxidative stress with dual functionality.

The synthesis of a molecule that unifies moieties mimicking superoxide dismutase and glutathione peroxidase is described. The hybrid delivers new opportunities as a potential agent against two significant reactive oxygen species, superoxide and hydrogen peroxide, playing a crucial role in various diseases.



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