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Chalcogen bonding interaction between ebselen and nitrite promote *N*-nitrosation of amines†

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Ebselen (EbSe), a therapeutically significant molecule, is shown to exhibit chalcogen bonding interaction with nitrite anion (ONO^-). This report suggests that the σ-holes of EbSe are powerful for offering weak but influential interactions towards biologically relevant ONO^-, thereby assisting oxidative transformations like *N*-nitrosation of aromatic amines.

Ebselen (**EbSe**) is a lipid-soluble organoselenium compound with remarkable therapeutic potential against a wide array of disease conditions related to the heart, tumour growth, inflammation, and many more.[1] The biological activities of **EbSe** are primarily attributed to its efficiency in extinguishing highly reactive oxidants (Fig. 1A).[2] For instance, **EbSe** exhibits glutathione peroxidase (GPx) activity,[3] thereby providing protection for various organisms against reactive oxygen species (ROS). GPx catalyses reduction of hydroperoxide (**ROOH**) in the presence of glutathione (GSH)/thiol cofactors (Fig. 1B). **EbSe** is also known to scavenge the incredibly reactive peroxynitrite anion ($ONOO^-$) mostly by transforming it to nitrite anion (ONO^-), along with the formation of the corresponding selenoxide species **EbSeO** through oxygen-atom transfer reactivity of peroxynitrite (Fig. 1C),[4] while **EbSe** catalysed isomerization of $ONOO^-$ to nitrate anion (ONO_2^-) has also been suggested in the literature.[5]

Understanding the interactions of **EbSe** with various reactive oxidants is of prime importance in order to utilize ebselen or related derivatives as an efficient mediator of the antioxidant activities in the biological milieu. The widely accepted mechanism for the GPx activity of **EbSe** indicates an initial nucleophilic attack of thiol GSH leading to the heterolytic cleavage of the Se–N bond in **EbSe** to produce the corresponding selenenyl sulfide **EbSeSG** as the intermediate species prior to the formation of the active reductant **EbSeH** in the presence of thiol (Fig. 1B).[3] Alternatively, the ROS like peroxide (**ROOH**) may directly interact with **EbSe** to yield selenoxide species **EbSeO** through a formal oxygen-atom transfer from the ROS, and thus may quench the oxidizing capacity of the ROS. A previous computational study on the reactions of **EbSe** and $ONOO^-$ suggests that an initial coordination of the peroxynitrite anion at the Se-site of **EbSe** is exothermic and yields a transient adduct complex **EbSe**···$OONO^-$ (Fig. 1C),[6] which subsequently promotes the cleavage of the O–O bond in **EbSe**···$OONO^-$ prior to its transformation to either (**EbSeO** + ONO^-) or (**EbSe** + ONO_2^-). It is noteworthy that the initial steps for all the above-mentioned mechanistic postulates involve the Se-site of **EbSe** as the LUMO features a σ*(Se–N) character with a predominant contribution from the Se-orbital (Fig. 2A).[7] Moreover, adduct complexes of **EbSe** chalcogen bonded

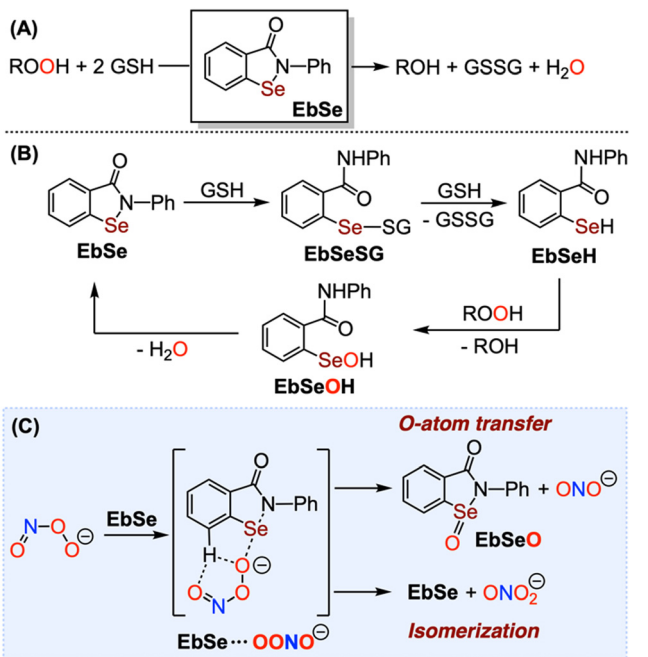

Fig. 1 (A) and (B) GPx activity of **EbSe** and a commonly proposed mechanism. (C) **EbSe** mediated transformations of the peroxynitrite anion ($ONOO^-$) *via* the corresponding adduct complex **EbSe**···$OONO^-$.

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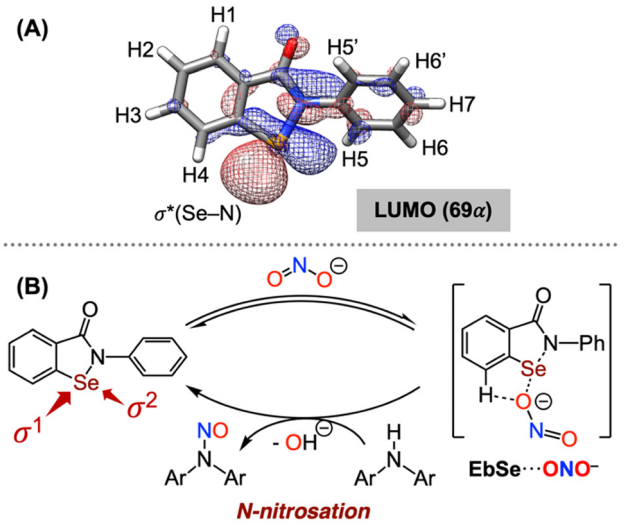

Fig. 2 (A) LUMO of ebselen calculated at the B3PW91/6-311G(2df,p) level of theory. (B) Overview of this work. The arrows indicate the location of σ-holes (σ^1 and σ^2).

to a variety of coordinating solvents (*e.g.* acetonitrile, tetrahydrofuran, dimethylsulfoxide) and pyridine derivatives have been illustrated earlier.[8–10] Notably, a previous study based on high resolution X-ray diffraction data as well as computational analyses on **EbSe** suggest that the Se-site contains two σ-holes (σ^1 and σ^2), along the σ*(Se–N) and σ*(Se–C) directions, respectively (Fig. 2B), which are capable of engaging in directional non-covalent Se···X (X = O, N) interactions.[11,12] Based on the computational studies, these σ-holes have been proposed to recognize physiologically relevant small molecular species such as H_2O, H_2O_2, $O_2^{\bullet-}$, and $^{\bullet}OH$ primarily through Se···O interaction. In fact, the previously proposed interactions of the Se-site in **EbSe** with peroxynitrite (**OONO**$^-$) or nitrate (**ONO$_2^-$**) perhaps originate from the noncovalent interactions at the Se-based σ^1-hole.[12]

While the interactions of **EbSe** with various ROS (such as peroxides, peroxynitrite, and hydroxyl radical) were previously assessed computationally,[4–6,11] the reactivity of nitrite anion (**ONO**$^-$) with ebselen (**EbSe**)/ebsulfur (**EbS**) is not known to the best of our knowledge. Inspired by our recent work on the generation of reactive intermediates from the reaction of nitrite anion and organosulfur compounds,[13] the objective of this work is to probe the interactions/reactions between **EbSe**/**EbS** and **ONO**$^-$ using in-depth NMR spectroscopic and computational analyses. This work reveals that the noncovalent interactions in the resultant adduct complex **EbSe**···**ONO**$^-$ amplify the reactivity of the otherwise stable nitrite anion towards aromatic amines (Fig. 2B).

Multinuclear (^{1}H, ^{13}C, ^{77}Se) NMR spectra of an authentic sample of **EbSe** and a solution consisting of an equimolar mixture of tetra-*n*-butylammonium nitrite **[TBA][ONO]** and ebselen (**EbSe**) in $CDCl_3$ at room temperature were compared for gaining insights into the interaction between **EbSe** and the **ONO**$^-$ anion. ^{77}Se NMR spectroscopic investigation on a 1 : 1 solution of **EbSe** and nitrite anion depicts a chemical shift at $\delta(^{77}\text{Se})$ = 942.19 ppm, whereas an authentic sample of **EbSe** shows $\delta(^{77}\text{Se})$ = 961.40 ppm (Fig. 3 and Fig. S1, ESI†). The

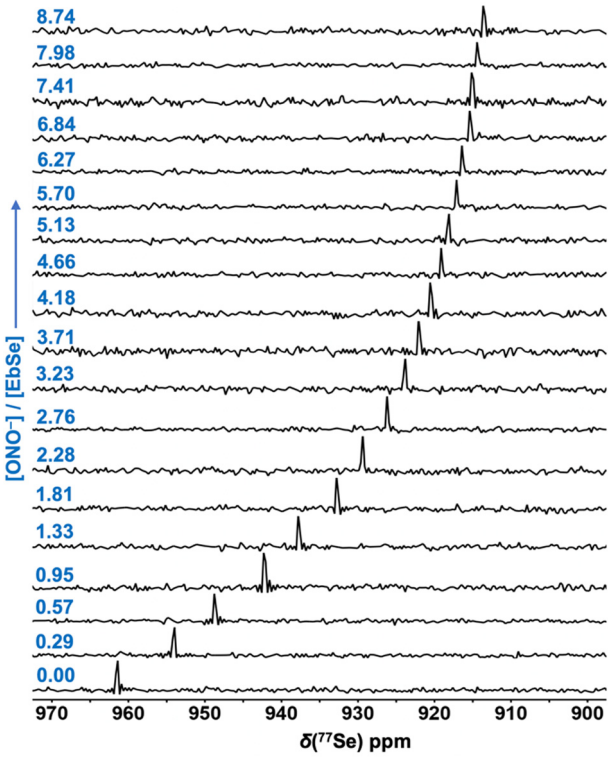

Fig. 3 ^{77}Se NMR (95 MHz) spectra of **EbSe** in $CDCl_3$ at room temperature upon increasing the ratio of nitrite anion and **EbSe** from 0.00 to 8.74 (as shown in the left panel).

upfield shift of the ^{77}Se resonance for **EbSe** originates due to Se···O interaction in the **EbSe**···**ONO**$^-$ adduct and this is consistent with the previously reported **EbSe** complexes chalcogen-bonded to the guest molecules.[8] Comparison of the ^{1}H and ^{13}C NMR spectra of the adduct complex **EbSe**···**ONO**$^-$ displays that both the proton and carbon resonances distinctly shift relative to those of an authentic sample of **EbSe** (Fig. S2 and S3, ESI†). Furthermore, NMR experiments such as ^{13}C DEPT135, ^{1}H–^{1}H COSY, and ^{1}H–^{13}C HMQC on **EbSe**···**ONO**$^-$ in $CDCl_3$ at room temperature allow us to assign the chemical shift of each aromatic proton individually (Fig. S4–S9, ESI†). While most of the proton resonances of **EbSe** undergo minor to moderate upfield shifts upon the coordination of the nitrite anion, the chemical shift of the H4-proton encounters a distinct downfield shift (Fig. 4 and Fig. S2, ESI†). Thus, these findings reveal that the nitrite anion is H-bonded to the H4–C4 site of **EbSe** in addition to the above-mentioned Se···O interaction.

^{77}Se and ^{1}H NMR spectra of **EbSe** with a systematic variation in the amount of added **[TBA][ONO]**, where [**ONO**$^-$]/[**EbSe**] ranged from 0.00 to 8.74, in $CDCl_3$ at room temperature were recorded (Fig. 3 and 4). The increasing amount of nitrite anion equivalent in the solution of **EbSe** shows a gradual upfield shift of the ^{77}Se resonance $\delta(^{77}\text{Se})$ (Fig. 3). A fitting of this shift in $\delta(^{77}\text{Se})$ with the 1 : 1 host–guest interaction model provides an association constant (K_a) of 25.22 M^{-1} ($\log K_a$ = 1.40) (Fig. S10, ESI†).[14,15] Notably, the association constant for **EbSe**···**ONO**$^-$ is moderately higher as compared to that of the previously reported **EbSe**···**HMPA** complex (HMPA = hexamethylphosphoramide) and this may be attributed to factors such as the anionic charge on the guest.[8] In order to

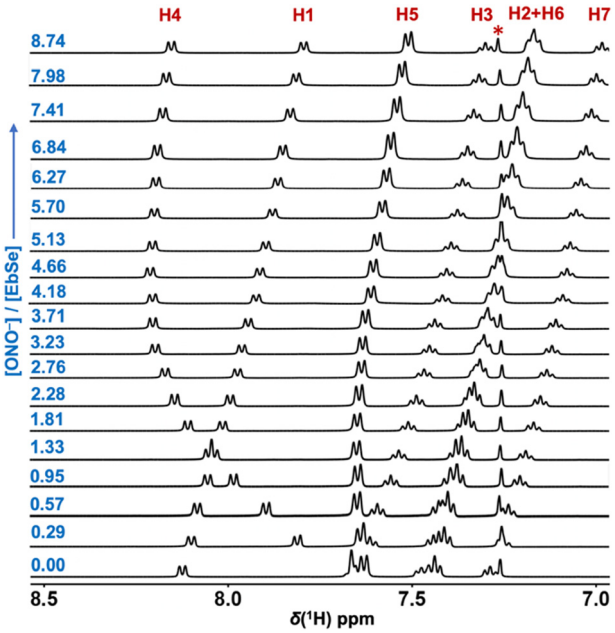

Fig. 4 ^{1}H NMR spectra of **EbSe** in $CDCl_3$ at room temperature upon increasing the ratio of nitrite anion and **EbSe** from 0.00 to 8.74 (as shown in the left panel). The peak marked with * originates from the $CDCl_3$ solvent residual peak. See Fig. 2A for the numbering scheme.

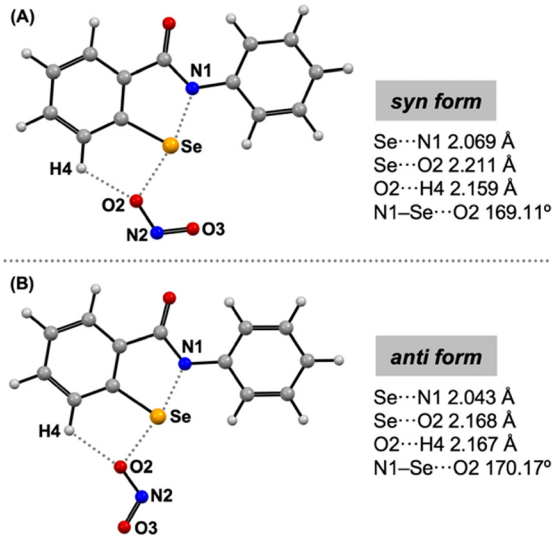

Fig. 5 DFT optimized (B3PW91/6-311G(2df,p)) structures of **EbSe**···**ONO**$^-$ in *syn*-form (A) and *anti*-form (B) with respect to the Se···O–N═O moiety.

understand the role of the coulombic charge of the guest molecule on ebselen adduct complexes, ^{77}Se and ^{1}H NMR studies were carried out on the solutions of tetra-*n*-butylammonium nitrate **[TBA][ONO$_2$]** and **EbSe** in $CDCl_3$ at room temperature. Both the ^{77}Se and ^{1}H NMR studies suggest very weak association between **EbSe** and **ONO$_2^-$** anion (Fig. S12 and S13, ESI†). Notably, nitrate is known to be poorly coordinating as compared to nitrite due to more delocalization of the negative charge.[16]

We turned to investigate the binding of nitrite anion with a sulfur analogue of **EbSe**, namely 2-(4-methylphenyl)-1,2-benzisothiazol-3(2*H*)-one (**EbS**). Unlike the LUMO of **EbSe**, the LUMO of **EbS** shows a minor contribution from σ*(S–N) (Fig. S14, ESI†).[7] A series of ^{1}H NMR spectra recorded on the samples consisting of **EbS** and varied equivalents of **[TBA][ONO]** in $CDCl_3$ at room temperature show negligible shifts of the proton resonances (Fig. S15, ESI†). Thus, this suggests that **EbS** is not an effective host for nitrite anions, as it is not capable of offering a strong S···O interaction with the weak σ-hole of a relatively lighter chalcogen site.

Unfortunately, several attempts to grow crystals of the adduct complex **EbSe**···**ONO**$^-$ failed due to the limited choice of suitable non-coordinating solvents. Hence, two possible isomers of **EbSe**···**ONO**$^-$ (*syn*/*anti*) and **EbSe**···**ONO$_2^-$** were calculated at the B3PW91/6-311G(2df,p) level of density functional theory (DFT) (Fig. 5 and S16, S17, ESI†).[7] The *syn*- and *anti*-isomers are nearly isoenergetic as the relative energy differs marginally by 2.18 kcal mol^{-1}. The DFT optimized structure of the *syn*-isomer of **EbSe**···**ONO**$^-$ depicts a Se···O2 interatomic distance of 2.211 Å along with an elongated Se–N1 bond (2.069 Å in *syn*-**EbSe**···**ONO**$^-$) in comparison to the Se–N1 bond length of 1.875 Å in free **EbSe** (Fig. 5A and Table S2, ESI†). Similarly, the *anti*-isomer also shows comparable metrical parameters (Se···O2 2.168 Å and Se–N1 2.043 Å). It is noteworthy that these Se···O interatomic distances in both the *syn*- and *anti*-isomers of **EbSe**···**ONO**$^-$ are significantly shorter in comparison to the sum of the van der Waals radii Se + O 3.42 Å (Fig. S16, ESI†), thereby suggesting noncovalent interactions between the Se-site and nitrite anion. Moreover, these Se···O distances are comparable to the Se···N distances ranging from 2.304(1) to 2.6166(15) Å obtained from the X-ray crystal structures of the adducts of **EbSe** with various N-bases.[9] In contrast to the notable Se–N1 bond elongation **EbSe**···**ONO**$^-$, changes in other bond lengths of **EbSe** upon the coordination of the nitrite anion are negligible. Moreover, the nearly linear N1–Se···O2 angle 169.11° and 170.17° for the *syn*- and *anti*-isomers indicates the directional nature of the chalcogen bonding interaction of the nitrite anion with the σ-hole along the more polar σ*(Se–N) direction,[12] thereby resulting in additional H-bonding interaction with the H4-site and consistent with the ^{1}H NMR studies (Fig. 3 and 4). Interaction of the nitrite anion with **EbSe** also results in a marginal elongation of the O2–N2 bond by 0.032 (/0.048) Å and contraction of the N2–O3 bond by 0.039 (/0.047) Å as observed for the *syn* (/*anti*) isomers of **EbSe**···**ONO**$^-$. The geometry optimized structure of **EbSe**···**ONO$_2^-$** shows a Se···O2 interatomic distance of 2.295 Å, which is longer than that in **EbSe**···**ONO**$^-$. Consequently, the Se–N1 distance of 1.990 Å in **EbSe**···**ONO$_2^-$** is shorter relative to that in **EbSe**···**ONO**$^-$ (Fig. 5 and Fig. S17, ESI†). Thus, these findings from the computational and NMR studies correlate well and unambiguously demonstrate that chalcogen bonding interaction in **EbSe**···**ONO**$^-$ is stronger than in **EbSe**···**ONO$_2^-$**.

Aiming to understand the implications of such chalcogen bonding interaction on the reactivity profile of the nitrite anion, *N*-nitrosation of amines in the presence and absence of **EbSe** was investigated. An equimolar reaction of **[TBA][ONO]** and di-*p*-tolylamine (**Tol$_2$NH**) in the presence of **EbSe** (1.0 equiv.) in

Table 1 Summary of the *N*-nitrosation reactions in dichloromethane

Entry	Reaction	Yield of *N*-nitrosamine (%)
1	[TBA][NO_2] + $(Tol)_2NH$ (**Control**)	7
2	[TBA][NO_2] + $(Tol)_2NH$ + **EbSe**	61
3	[TBA][NO_2] + $(Tol)_2NH$ + **EbS**	37
4	[TBA][NO_2] + $(Tol)_2NH$ + **EbSe** (10 mol%)	52
5	2 [TBA][NO_2] + $(Tol)_2NH$ + **EbSe** (10 mol%)	100
6	[TBA][NO_2] + 2 $(Tol)_2NH$ + **EbSe** (10 mol%)	48
7	[TBA][NO_2] + $(Tol)_2NH$ + **EbS** (10 mol%)	28
8	[TBA][NO_2] + PhNHMe + **EbSe**	91
9	[TBA][NO_2] + $^{i}Pr_2NH$ + **EbSe**	Trace

dichloromethane at room temperature provides the corresponding *N*-nitrosamine **Tol_2NNO** in 61% yield (Table 1, Fig. S18–S20, ESI†). Notably, a control reaction of **[TBA][ONO]** and **Tol_2NH** under the analogous reaction conditions in the absence of **EbSe** yields a trace (7%) amount of **Tol_2NNO**. Interestingly, the above-mentioned reaction in the presence of **EbSe** in coordinating solvents like acetonitrile or tetrahydrofuran does not provide **Tol_2NNO**. Thus, these observations suggest that (a) the activation of nitrite through the chalcogen bonding interaction with **EbSe** is critical for *N*-nitrosation of amine; (b) coordinating solvents compete for the chalcogen bonding interactions, thereby hindering the activation of the nitrite anion and inhibiting *N*-nitrosation. Similarly, inhibition by a product like *N*-nitrosated amine cannot be ruled out. A reaction of **[TBA][ONO]** and **Tol_2NH** (1 : 1) in the presence of **EbSe** (10 mol%) in dichloromethane gives 52% yield of **Tol_2NNO** (considering nitrite as the limiting reagent). A comparison of the yields of **Tol_2NNO** upon systematic variation of either nitrite anion or amine equivalents keeping **EbSe** loading fixed demonstrates that (a) the yield of the reaction drops in the presence of higher equivalent of amine; (b) higher equivalent of the nitrite anion enhances the efficiency of *N*-nitrosation. While arylamines (such as di-*p*-tolylamine and *N*-methylphenyl amine) efficiently undergo *N*-nitrosation in the presence of nitrite anion and **EbSe** (Fig. S21, ESI†), aliphatic amine (*e.g.* diisopropylamine) provides a trace yield under the analogous reaction conditions. This may be attributed to the higher basicity of the aliphatic amines, thereby competing against the nitrite anion in interacting with **EbSe**. Thus, these findings indicate that the proposed *in situ* generated **EbSe···ONO$^-$** adduct complex is capable of catalysing *N*-nitrosation reaction, while competitive binding of the amine substrate may impede the reaction outcome. This activation of the nitrite anion at the Se-site of **EbSe** and subsequent *N*-nitrosation are somewhat reminiscent of the nitrosating reactivity of the nitrite towards nucleophiles in the presence of Lewis acidic metal sites.[17] Gutmann–Beckett analysis to evaluate the Lewis acidic nature of **EbSe**, however, does not suggest any considerable Lewis acidic nature of **EbSe** (Fig. S22, ESI†).[18]

In conclusion, this work provides spectroscopic illustrations of various weak interactions between two physiologically significant molecular entities, namely nitrite anion (**ONO$^-$**) and ebselen **EbSe**, while such experimental demonstration of **EbSe** interaction was previously limited to various stable guests like coordinating solvents and N-bases.[8–10] Though weak in nature, the Se-centered σ-hole interaction in **EbSe···ONO$^-$** is shown to be effective in promoting aromatic amine oxidation leading to *N*-nitrosamine, a potential carcinogen.[19] Of broader significance, this work underscores that ebselen may be capable of activating biologically relevant oxidants and mediate oxidative modifications of the amino acids in the absence of a redox cofactors like thiol.

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Data availability

The data supporting this article have been included as part of the ESI.†

Conflicts of interest

There are no conflicts to declare.

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