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J. Am. Chem. Soc., **Just Accepted Manuscript** • DOI: 10.1021/jacs.7b06968 • Publication Date (Web): 19 Sep 2017

HNO is produced by the reaction of NO with thiols

Sebastian A. Suarez,[†,‡] Martina Muñoz,[†,‡] Lucia Alvarez,[†] Mateus F. Venâncio,[†] Willian R. Rocha,[†] Damian E. Bikiel,[†] Marcelo A. Marti[*,▪] and Fabio Doctorovich.[*,†]

[†] Departamento de Química Inorgánica, Analítica y Química Física, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, Buenos Aires, Argentina. INQUIMAE-CONICET, Ciudad Universitaria, Buenos Aires, Argentina [†] Universidade Federal de Minas Gerais, Departamento de Química, Belo Horizonte, Brasil [▪] Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, Buenos Aires, Argentina. IQUIBICEN-CONICET, Ciudad Universitaria, Buenos Aires, Argentina.

KEYWORDS: Nitric Oxide, thiols, nitroxyl, HNO, azanone

ABSTRACT: Azanone (nitroxyl, HNO) is a highly reactive compound whose biological role is still a matter of debate. One possible route for its formation is NO reduction by biological reductants. These reactions have been historically discarded due to the negative redox potential for the NO,H+/HNO couple. However, the NO to HNO conversion mediated by vitamins C, E and aromatic alcohols has been recently shown to be feasible from a chemical standpoint. Based on these precedents we decided to study the reaction of NO with thiols as potential sources of HNO. Using two complementary approaches, trapping by a Mn porphyrin, and an HNO electrochemical sensor, we found that under anaerobic conditions aliphatic and aromatic thiols (as well as selenols) are able to convert NO to HNO, albeit at different rates. Further mechanistic analysis using ab-initio methods show that the reaction between NO and the thiol produces a free radical adduct RSNOH*, which reacts with a second NO molecule to produce HNO and a nitrosothiol. The nitrosothiol intermediate reacts further with RSH to produce a second molecule of HNO and RSSR, as previously reported.

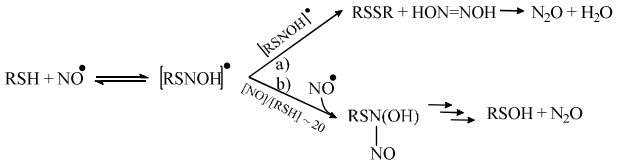
HNO (nitroxyl, or azanone), is an increasingly important biochemical active molecule, whose elusive nature originates in its extremely fast dimerization (k_{dim} = 8 x 10^6 $M^{-1}s^{-1}$), that limits its maximum concentration and life time.[1] It also reacts fast with NO (k = 5.6 x 10^6 $M^{-1}s^{-1}$),[2] thiols (k = 3 x 10^6 $M^{-1}s^{-1}$),[3–8] metalloproteins (k = 0.5 – 5 x 10^5 $M^{-1}s^{-1}$) [9–13] and at moderate rate with oxygen (k= 3 x 10^3 $M^{-1}s^{-1}$).[1,14,15] Given the mentioned instability, azanone donors are used in most (bio)chemical studies.[19,20] The biological relevance of HNO has two main aspects. The first one concerns the study of its pharmacological effects - mainly related with the cardiovascular system, including the treatment of heart failure[16–18] and the elucidation of its overlapping and differential reactivity with NO. The second aspect, and most relevant in the present case, concerns the possibility of its endogenous production.[19,21] Enzymatic proposed physiological relevant routes to HNO, concern oxidation of arginine,[21–23] or the reaction of NO[21] or nitrosyl species[24] with H_2S.[25] Although they make a strong point for enzymatic HNO production, it should be stressed that none of them has been confirmed *in-vivo*.[19]

The chemical (non-enzymatic), biological compatible, routes to HNO have been much less pursued.[26] The most direct route, chemical reduction of NO, has been historically discarded, possibly due to the negative reduction potential of –0.8 V proposed for the NO/^{3}NO$^-$ couple.[27] However, at physiological pH, ^{1}HNO is expected to be the main species with an estimated E^o (NO,H$^+$/^{1}HNO) ≈ −0.11 V, becoming −0.55 V at pH 7.[27] Furthermore, this value is nowadays under revision: recently Rocha *et. al.* computed E^o (NO,H$^+$/^{1}HNO) ≈ -0.16 V at pH 7 in aqueous solution, using quantum mechanical calculations and Monte Carlo statistical mechanical simulations.[28] Moreover, the reduction of NO to HNO is necessarily coupled to other reactions that produce compounds such as N_2O, which could drive the reaction forward, overcoming an unfavorable thermodynamic barrier.[29,30] This chemical reductive route recently received key support, showing that NO can be converted to HNO by hydrogen sulfide (H_2S)[25] or aromatic alcohols (i.e. ascorbic acid, tyrosine).[29]

H-atom abstraction from thiols could in principle be another reductive pathway for HNO formation. Early studies showed that NO ‘‘slowly’’ reacts with thiols leading to formation of disulfides, N_2O and eventually N_2.[31,32] Pryor and coworkers found that anaerobic aqueous solutions of thiophenol (PhSH) and thiols exposed to NO, result in quantitative formation of the corresponding disulfides (RSSR).[32] This reaction becomes faster to completion as the pH gets closer to the pKa of the thiol. However, HNO for mation was ruled-out due to the observation of base catalysis, despite N_2O being a major reaction product. A few years later, Nagasawa and coworkers,[7,33] studied the anaerobic reaction of albumin (a thiol-containing protein) and other biological thiols such as glutathione with large excess (> 10 times) of NO. These reactions, which took place in minutes, were shown to produce N_2O, and the corresponding sulfenic acids RS-OH were proposed as intermediates. In both works the initial formation of a thionitroxide radical RSNOH$^\bullet$ was suggested (Scheme 1), but while Pryor proposed dimerization of the free radical leading to the disulfide, Nagasawa suggested that it could react with excess NO, leading to an N-nitroso intermediate that would yield the sulfenic acid and N_2O. The rate constant for the anaerobic reaction of NO with cysteine at 25 °C was determined to be 3.7 x 10^2 $M^{-1}s^{-1}$,[34] assuming the mechanism proposed

by Pryor.[32] The fact that these authors disregarded HNO as a reaction intermediate, possibly originates in a lack of precise detection tools for HNO, being all indirect at the moment (between 1982 and early 2000). In any case, the presence of N_2O as a final product and the observed NO to HNO conversion mediated by alcohols suggest that the reaction of thiols with NO could involve HNO as an intermediate.

Scheme 1.



Another important point is related to the formation of nitrosothiols (RSNO) and their subsequent reactions, yielding disulfides and HNO.[6] On the other hand, HNO reacts with thiols at a fast rate (ca 10^6 $M^{-1}s^{-1}$) yielding a thiol-bound hydroxylamine that decays to several thiol oxidized species including predominantly the disulfide.[35] N_2O release from the reaction of GSNO and GSH was detected ($k \approx 10^{-2}$ $M^{-1}s^{-1}$),[6] even in the presence of 10 equivalents of GSH. This observation implies that it is possible to detect HNO in the presence of an excess of thiol, although it is generated in a "slow" manner and reacts ''quickly'' with the free thiol.

Therefore, in this complex scenario of cross reactions between thiols, NO and HNO, it is of primordial relevance to be able to detect and quantify HNO *in-situ* and reliably in small concentrations to assess its presence unequivocally and understand the underlying reaction mechanisms. For this sake, in the present work we investigated the rate of HNO production from the reaction of NO with thiols.

We begin our analysis of the reactions of NO with 1-hexanethiol (R_6SH), cysteine (Cys), benzenethiol (Ph-SH), and benzeneselenol (Ph-SeH), using Mn(III)TCPP as a trapping agent for HNO.[36] Figure 1A (inset) shows the absorbance changes observed upon addition of Cys to a solution of NO containing Mn(III)TCPP, which are indicative of porphyrin conversion to Mn(NO)TCPP due to HNO production (no reaction with the porphyrin, i.e no spectral changes, are observed either with the addition of NO or any of the thiols alone during the experimental timescale). Figure 1A shows that the amount of trapped HNO, as revealed by the growth in nitrosylporphyrin (Abs_{424nm}), increases with time and is larger for higher Cys concentrations. Similar results are observed for the other thiols and selenol (Figure 1B), although the rate and amount of produced HNO varies in the following order: Ph-SeH > Ph-SH >> Hex-SH > Cys. This order is possibly related to the decreasing stability of the corresponding $RSe^{\bullet}$ or $RS^{\bullet}$ free radicals (see below).

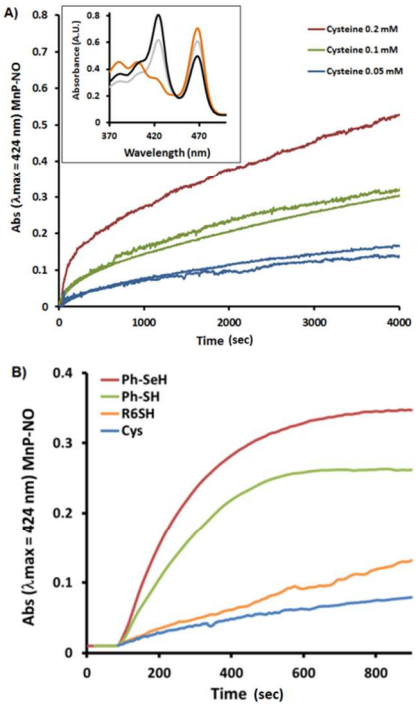


Figure 1. A) [Mn(NO)TCPP] produced after mixing 2 mM NO with Cys 50 μM (blue), 100 μM (green) and 200 μM (red), duplicated runs. Inset: [Mn(NO)TCPP] (black, t= 4000 sec; grey, t=1500 sec) produced as a function of time by mixing NO (2 mM) with Cys (200 μM) in the presence of Mn(III)TCPP (1 μM, orange, t=0 sec). B) [Mn(NO)TCPP] produced after mixing 2 mM of NO with 50 μM of each thiol: Cys (blue), R_6SH (orange), Ph-SH (green) and Ph-SeH (red). The reaction starts when the thiols are added (t=100 sec).

To have an independent confirmation of HNO production and in order to determine the corresponding reaction rates, we measured the time courses of [HNO] for the above described reactions using an HNO selective electrode.[37,38] Figure 2A shows the [HNO] vs. time plot obtained after addition of Cys to an NO solution, clearly evidencing how the electrode signal raises rapidly, revealing the presence of produced HNO (see the other compounds in SI). Figure 2B shows that the initial HNO production rate (v_i) scales linearly with both R_6SH (orange) and NO (gray) concentrations, while the other reactant concentration is maintained constant and in excess. From these plots an effective bimolecular reaction rate constant (k_{eff}), corresponding to equation 1, can be determined. Finally, the corresponding log vs. log plots (shown in SI) confirm that the reaction is first order in both reactants.

$$v = d[HNO]/dt = k_{eff}[NO][RSH] \qquad (1)$$

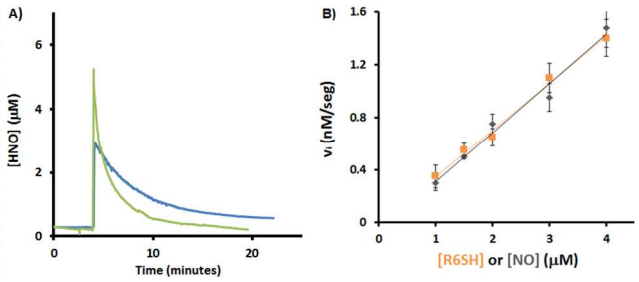


Figure 2. A) In blue, [HNO] determined electrochemically after addition of Cys (20 μM) to an anaerobic solution of 200 μM NO in the presence of DPTA 10^{-4} M. k_{obs}: is determined from the slope of the electrode signal vs time plots. In green, [HNO] after simultaneous addition of Cys (20 μM) and O_2 (20 μM) to an anaerobic solution of 200 μM NO. B) v_i vs [R_6SH] or [NO], while the other reactant concentration is maintained constant (10 μM).

Similar results were obtained for Ph-SH and Ph-SeH (see Figure SI3 and SI4). The resulting effective rate constants reported in Table 1 show that Cys and R_6-SH, both aliphatic thiols, display similar rates, about 4 times smaller than those observed for Ph-SH and Ph-SeH. It should be taken into account that the rate constants of HNO production ($k_{HNO-RSH}$) could be significantly higher, because the obtained k_{eff}s also comprise HNO consumption (i.e. HNO reaction with NO, see below).

We also tested whether O_2, Fe(II), Mn(II) and Cu(II) affected HNO production in the described reactions. Electrochemical [HNO] measurements were performed in both the absence and presence of various metal ions (final added concentration of 0.02 mM). Figure 3 shows the relative peak [HNO] with respect to the ion free reaction (performed in the presence 0.1 mM DPTA as chelating agent) for the three tested ions Cu(II) Fe(II) and Mn(II). As shown in Figure 3, there is no catalytic effect of iron, consistent with previous results.[39] Copper has been shown to catalyze the production of RS* and accordingly, there is an increase in the peak for [HNO], but the increase is moderate (twofold). The results for Mn are in between. .

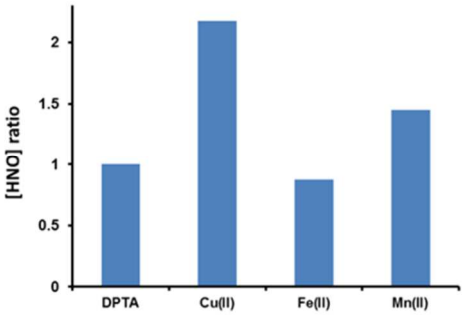


Figure 3. Relative HNO production in the described reactions by using the electrochemical nitroxyl sensor in the presence of Fe(II), Mn(II) and Cu(II), with respect to the ion free reaction.

When O_2 was added to the reaction in 1:1 ratio with respect to Cys (see Figure 2A – green line, Table SI2), faster production of HNO was observed. This is consistent with the rapid formation of nitrosothiol in the presence of O_2, as previously described,[40] and subsequent reaction of RSNO with RSH to produce HNO.[6] However, the rate and amount of produced HNO decreases as the amount of added O_2 is increased by ten times or more, as previously shown.[29]

Table 1. k_{eff} and N_2O - nitrite ratio obtained for the reactions of thiols with NO (pH = 7.4, rt, anaerobic, in the presence of DPTA).

Compound	k_{eff} ($M^{-1}s^{-1}$)[a]	NO_2^- :N_2O[b]	Ref
Ph-SeH	125 ± 5 (115)	0.9 ± 0.1	This work
Ph-SH	110 ± 8 (105)	1.0 ± 0.1	This work
R_6-SH	35 ± 5 (32)	0.9 ± 0.1	This work
Cys	25 ± 6 (20)	1.2 ± 0.1	This work
Cys	370*	-	34
BSA[c]	0.6	-	34
Ascorbic Acid	8	1.2	29
Hydroquinone	6	1.2	29
Tyrosine	1	1.2	29

The high reactivity of reactants and products results in a complex mechanistic scheme.[29,30] Therefore, to analyze possible mechanisms we quantified several products, especially those that have not been previously characterized.[32] The first important reaction to consider (Eq. 2), is that between NO and HNO shown below (k = 6 x 10^6 $M^{-1}s^{-1}$).[2]

$$2\ NO + HNO \rightarrow\rightarrow\rightarrow NO_2^- + N_2O \qquad (2)$$

For each reaction, the final concentrations of nitrite in solution, and the amount of N_2O in the reaction chamber headspace were determined (see SI).[29,37] The results presented in Table 1 show that in all cases N_2O and NO_2^- are produced in approximately a 1:1 ratio, as expected from eq. 2. Reaction of HNO with thiols could lead to further reduced species such as hydroxylamine or even ammonia.[6] NH_3 was quantified by acid/base titration and its yield is ca. 20-35% based on the total initial amount of NO (see SI). Hydroxylamine was not detected (see SI). Accordingly, NH_2OH is expected to be further reduced to NH_3 (Eqs. 3 and 4).[6,41]

$$RSH + RSNO \rightarrow RSN(OH)SR \qquad (3)$$

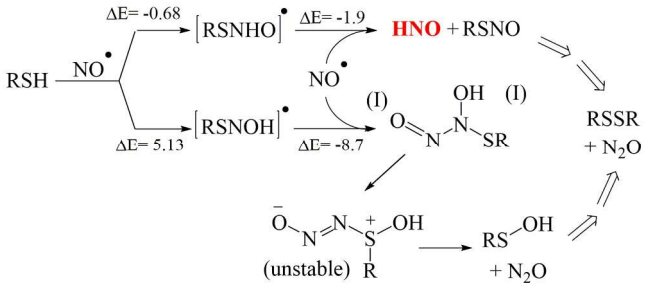
$$RSN(OH)SR + 4RSH \rightarrow\rightarrow\rightarrow RSSR + H_2O + NH_3 \qquad (4)$$

The sum of the recovered nitrogen yield based on the total amount of reacting NO varies from 61% in the case of Cys to 96% for Ph-SeH after 2 h of reaction (4-9% of NO was left unreacted). We also used NMR spectroscopy to characterize the thiol-derived end products. In all cases the disulfides were observed, i.e cystine, diphenyl disulfide - as shown in previous works,[32] di-N-hexyl disulfide, and diphenyl diselenide.

To gain a deeper understanding of the studied reaction we used ab-initio methods to determine the energetics and structures of all possible reaction steps and species, taking as start-

ing points the proposals by Pryor and Nagasawa[32,33] as well as our previous work with aromatic alcohols.[29,30] The results presented in Scheme 2, and SI, show that the first step comprises a proton-coupled nucleophilic attack (PCNA) of the thiol to NO,[29,30] that yields a thionitrite radical intermediate $RSNO(H)^{\bullet}$.

Scheme 2.



By using hydration with Zundel ions it can be shown that the reaction varies from slightly exergonic to endergonic depending on the protonation site (-0.68 to +5.13 kcal/mol for R=CH_3 and -2.51 to 1.88 kcal/mol for R=Ph), and that the radical has two tautomers: one with the proton on the oxygen atom and the other, slightly more stable, on the nitrogen atom. For R = Ph (Figure 4), the first step barrier shows a value of 19.67 Kcal/mol, which is in reasonable agreement to the one predicted using the kinetic constant k_{eff} (ca. 15 Kcal/mol). Similar results were obtained using open shell DFT calculations with polarizable continuum model (see SI).

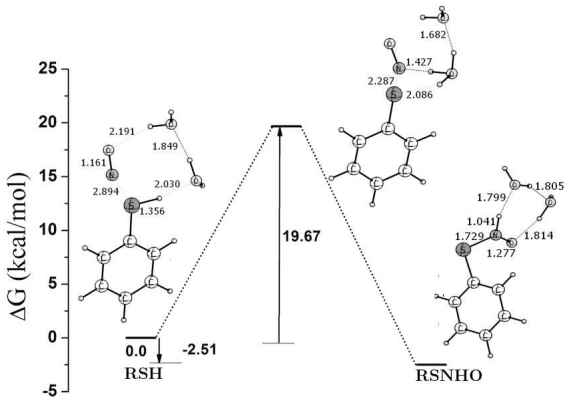


Figure 4. A) Proton-coupled nucleophilic attack of the RSH to the nitrogen atom of NO, mediated by a Zundel ion (R = Ph). The transition state has an imaginary frequency of 510.03i cm^{-1} and the nuclear motion associated with this mode is the concerted motion of the hydrogen atoms from the S-H group to the water and from the water to the nitrogen of the NO molecule, forming the N-H bond. Results are in kcal/mol

Direct decomposition of $RSNO(H)^{\bullet}$ to yield HNO and a thiyl radical $RS^{\bullet}$ is highly unfavorable (>30 kcal/mol), therefore the thionitrite is expected to react rapidly with a second molecule of NO, yielding either di-NO-thiol intermediates (species (I) in Scheme 2) or HNO and RSNO.[42] The decay of these di-NO-thiol intermediates to produce RSNO and HNO is endergonic (7.3 kcal/mol). If an excess of thiol is present, RSNO can react with a second molecule of thiol to yield disulfide and HNO, as previously suggested (see SI).[43] Last but not least, as proposed by Nagasawa,[33] another alternative is rearrangement of the O-protonated di-NO-thiol (I), yielding an intermediate sulfenic acid and N_2O. Besides, a possible HNO producing chain reaction mechanism is shown in SI.

Our results show that the reaction between NO and thiols generates HNO. The effective bimolecular rates (reported in Table 1) show that cysteine produces HNO about 3 times faster than the fastest aromatic alcohol -ascorbic acid-, and that the aromatic Ph-SH is even faster. To understand the relative reactivity and overall HNO yield two important issues stand out. First, as for the aromatic alcohols, the key and possible rate limiting step concerns formation and stability of the first radical intermediate, which in this case is the thionitrite radical $RSNO(H)^{\bullet}$. This intermediate is only stable after proton migration to either the O or N atoms, which allows the radical to be localized on the X-S (X=Cys, Ph, R_6) and not on the nitroso group. Aromatic groups allow for better stabilization of the unpaired spin, and thus show faster effective rates. Concerning the fate of $RSNO(H)^{\bullet}$, several possibilities need to be considered. Spontaneous decay to HNO and the thiyl radical is endergonic and is rendered unlikely. A clear difference when compared to alcohols such as ascorbic acid or hydroquinone which mainly react by this route. Dimerization of the radical -as proposed by Pryor[34] is expected to be slow due to its low effective concentration. Instead, reaction of the radical with a second molecule of NO is more likely under the present conditions (relatively high NO concentration), yielding HNO and RSNO. Interestingly, in the presence of excess thiol, RSNO reacts to yield another molecule of HNO and the disulfide, which is the main observed S containing organic end product. In this route, two molecules of one-electron reductants -the thiols- behave as one molecule of two-electron reductant -like ascorbic acid- according to the overall reaction (Eq. 5):

$$2NO + 2RSH \rightarrow 2HNO + RSSR \qquad (5)$$

The second point is related to thermodynamics, particularly to the reduction of NO to HNO. Recently, $E°(NO,H^+/HNO)$ was calculated by state of the art computational methods, rendering the value -0.16 V,[10] while that for $(RSSR,2H^+/2\ RSH)$ is close to -0.25 V,[29] and thus an outer sphere electron transfer is in principle thermodynamically favorable. Moreover, under conditions where NO is physiologically produced (or added pharmacologically) the relative [NO]/[HNO] ratio is expected to be over 1000, resulting in an effective E of ca. 0 V for the $(NO,H^+ / HNO)$ couple, amenable to be reduced by biological reductants such as cysteine, glutathione, FAD, NAD, tyrosine and vitamin C, *inter alia.*

In summary, the present work provides clear evidence for the reaction of NO with several thiols, as an HNO source. The reactions of NO with thiols have been studied for over thirty years yielding a rich picture of chemical reactivity which is still far from being completely understood. In this context, HNO emerges as a new reaction intermediate and a key player of this complex and highly reactive environment. The relationship between HNO and thiols is dual: thiols could be involved in HNO formation but they are also the main targets

for HNO bioactivity. Due to the high reactivity of HNO, it is expected that its steady state concentration inside cells should be several orders of magnitude lower than that of NO. Therefore the "physiological" redox potential for the ($NO, H^+/HNO$) couple is probably even more positive than that of ($O_2/O_2^{\bullet-}$), i.e. easily accessible. With the expanding arsenal of analytical tools for HNO detection[36,38,45–47] both in solution and in cells, there is no doubt that we are getting closer to understand the *in vivo* chemistry of HNO.

ASSOCIATED CONTENT

Kinetic analysis, ab initio calculations, and other experimental details can be found in the Supporting Information. This material is available free of charge via the Internet at http://pubs.acs.org

AUTHOR INFORMATION

Corresponding Author

* doctorovich@qi.fcen.uba.ar
* marcelo@qi.fcen.uba.ar

Author Contributions

The manuscript was written through contributions of all authors. / All authors have given approval to the final version of the manuscript. / ‡These authors contributed equally.

Funding Sources

This work was financially supported by UBA (UBACYT 20020130100642BA, 20020150100023BA), ANPCyT (PICT 2014-1278, PICT 2015-3854, and by the Bunge y Born Foundation.

Notes

The authors declare no competing financial interests.

ACKNOWLEDGMENT

SAS, MM and LA thank UBA & CONICET for a fellowship grant, SAS, DEB, MAM and FD are members of CONICET. MFV and WRR would like to thank the CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico, INCT-Catálise) and FAPEMIG (Fundação de Amparo à Pesquisa do Estado de Minas Gerais) for financial support and research grant.

REFERENCES

(1) Shafirovich, V.; Lymar, S. V. *Proc. Natl. Acad. Sci. U. S. A.* **2002**, *99*, 7340–7345.
(2) Lymar, S. V; Shafirovich, V.; Poskrebyshev, G. A. *Inorg. Chem.* **2005**, *44*, 5212–5221.
(3) Doyle, M. P.; Mahapatro, S. N.; Broene, R. D.; Guy, J. K. *J. Am. Chem. Soc.* **1988**, *110*, 593–599.
(4) Bartberger, M. D.; Fukuto, J. M.; Houk, K. N. *Proc. Natl. Acad. Sci. U. S. A.* **2001**, *98*, 2194–2198.
(5) Sherman, M. P.; Grither, W. R.; McCulla, R. D. *J. Org. Chem.* **2010**, *75*, 4014–4024.
(6) Wong, P. P. S.-Y.; Hyun, J.; Fukuto, J. M. J.; Shirota, F. N.; DeMaster, E. G.; Shoeman, D. W.; Nagasawa, H. T. *Biochemistry* **1998**, *37*, 5362–5371.
(7) Shoeman, D. W.; Shirota, F. N.; DeMaster, E. G.; Nagasawa, H. T. *Alcohol* **2000**, *20*, 55–59.
(8) Keceli, G.; Moore, C. D.; Toscano, J. P. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 3710–3713.
(9) Ford, P. C. P. C. *Inorg. Chem.* **2010**, *49* , 6226–6239.
(10) Hoshino, M.; Laverman, L.; Ford, P. C. *Coord. Chem. Rev.* **1999**, *187*, 75–102.
(11) Suárez, S. A.; Martí, M. A.; De Biase, P. M.; Estrin, D. a.; Bari, S. E.; Doctorovich, F. *Polyhedron* **2007**, *26*, 4673–4679.
(12) Martí, M. A.; Bari, S. E.; Estrin, D. A.; Doctorovich, F. *J. Am. Chem. Soc.* **2005**, *127*, 4680–4684.
(13) Boron, I.; Suárez, S. A.; Doctorovich, F.; Martí, M. A.; Bari, S. E. *J. Inorg. Biochem.* **2011**, *105*, 1044–1049.
(14) Miranda, K. M.; Dutton, A. S.; Ridnour, L. a; Foreman, C. a; Ford, E.; Paolocci, N.; Katori, T.; Tocchetti, C. G.; Mancardi, D.; Thomas, D. D.; Espey, M. G.; Houk, K. N.; Fukuto, J. M.; Wink, D. a. *J. Am. Chem. Soc.* **2005**, *127*, 722–731.
(15) Liochev, S. I.; Fridovich, I. *Free Radic. Biol. Med.* **2003**, *34*, 1399–1404.
(16) Ma, X. L.; Gao, F.; Liu, G. L.; Lopez, B. L.; Christopher, T. A.; Fukuto, J. M.; Wink, D. A.; Feelisch, M. *Proc. Natl. Acad. Sci. U. S. A.* **1999**, *96*, 14617.
(17) Ma, X. L.; Weyrich, A. S.; Lefer, D. J.; Lefer, A. M. *Circ. Res.* **1993**, *72*, 403–412.
(18) Switzer, C. H.; Flores-Santana, W.; Mancardi, D.; Donzelli, S.; Basudhar, D.; Ridnour, L. a; Miranda, K. M.; Fukuto, J. M.; Paolocci, N.; Wink, D. a. *Biochim. Biophys. Acta* **2009**, *1787*, 835–840.
(19) Doctorovich, F.; Bikiel, D.; Pellegrino, J.; Suárez, S. A.; Larsen, A.; Martí, M. A. *Coord. Chem. Rev.* **2011**, *255*, 2764–2784.
(20) Fukuto, J. M.; Bartberger, M. D.; Dutton, A. S.; Paolocci, N.; Wink, D. a; Houk, K. N. *Chem. Res. Toxicol.* **2005**, *18*, 790–801.
(21) Doctorovich, F.; Bikiel, D. E. D. E.; Pellegrino, J.; Suárez, S. A. S. A.; Martí, M. A. M. A. In *Progress in Inorganic Chemistry*; Kenneth D. Karlin, John Wiley & Sons, Inc, 2014.
(22) Stoll, S.; NejatyJahromy, Y.; Woodward, J. J.; Ozarowski, A.; Marletta, M. A.; Britt, R. D. *J. Am. Chem. Soc.* **2010**, *132*, 11812–11823.
(23) Donzelli, S.; Graham, M.; Flores-Santana, W.; Switzer, C. H.; Yeh, G. C.; Huang, J.; Stuehr, D. J.; King, S. B.; Miranda, K. M.; Wink, D. A.; Espey, M. G.; Flores-Santana, W.; Switzer, C. H.; Yeh, G. C.; Huang, J.; Stuehr, D. J.; King, S. B.; Miranda, K. M.; Wink, D. A. *Free Radic. Biol. Med.* **2008**, *45*, 578–584.
(24) Filipovic, M. R. M.; Miljkovic, J. L. J.; Nauser, T.; Royzen, M.; Klos, K.; Shubina, T.; Koppenol, W. H.; Lippard, S. J.; Ivanović-Burmazović, I.; Ivanovic, I. *J. Am. Chem. Soc.* **2012**, *134*, 12016–12027.
(25) Eberhardt, M.; Dux, M.; Namer, B.; Miljkovic, J.; Cordasic, N.; Will, C.; Kichko, T. I.; de la Roche, J.; Fischer, M.; Suárez, S. A.; Bikiel, D.; Dorsch, K.; Leffler, A.; Babes, A.; Lampert, A.; Lennerz, J. K.; Jacobi, J.; Martí, M. A.; Doctorovich, F.; Högestätt, E. D.; Zygmunt, P. M.; Ivanovic-Burmazovic, I.; Messlinger, K.; Reeh, P.; Filipovic, M. R.; Roche, J. de la; Fischer, M.; Bikiel, D.; Suárez, S. A.; Dorsch, K.; Leffler, A.; Babes, A.; Lampert, A.; Lennerz, J. K.; Jacobi, J.; Martí, M. A.; Doctorovich, F.; Högestätt, E. D.; Zygmunt, P. M.; Ivanovic-Burmazovic, I.; Messlinger, K.; Reeh, P.; Filipovic, M. R. *Nat. Comm.* **2014**, *5*, 4381.
(26) Kytzia, A.; Korth, H.-G.; Sustmann, R.; Groot, H. De; Kirsch, M.; de Groot, H.; Kirsch, M. *Chemistry* **2006**, *12*, 8786–8797.
(27) Flores-Santana, W.; Salmon, D. J.; Donzelli, S.; Switzer, C. H.; Basudhar, D.; Ridnour, L.; Cheng, R.; Glynn, S. A.; Paolocci, N.; Fukuto, J. M.; Miranda, K. M.; Wink, D. A. *Antioxid. Redox Signal.* **2011**, *14*, 1659–1674.
(28) Venâncio, M.; Doctorovich, F.; Rocha, W. *J Phys Chem B.* **2017**, *121*, 6618–6625.
(29) Suarez, S. A.; Neuman, N.; Muñoz, M.; Alvarez, L.; Brondino, C.; Bikiel, D. E.; Martí, M. A.; Doctorovich, F. *J. Am. Chem. Soc* **2015**, *137*, 4720–4727.
(30) Hamer, M.; Suarez, S. A.; Neuman, N. I.; Alvarez, L.; Muñoz, M.; Marti, M. A.; Doctorovich, F. *Inorg. Chem.* **2015**, *54*, 9342–9350.
(31) Reihlen, H.; Friedolsheim, A. V.; Oswald, W. *Justus Liebigs Ann Chem* **1928**, *465*, 72–96.
(32) Pryor, W.; Church, D.; Govindan, C. *J. Org.* **1982**, *V* (1), 156–159.
(33) DeMaster, E. G.; Quast, B. J.; Redfern, B.; Nagasawa, H. T. *Biochemistry* **1995**, *34*, 11494–11499.
(34) Aravindakumar, C. T.; Ley, M. De; Ceulemans, J. *J. Chem. Soc. Perkin Trans. 2* **2002**, 3, 663–669.
(35) Jackson, M. I.; Han, T. H.; Serbulea, L.; Dutton, A.; Ford, E.; Miranda, K. M.; Houk, K. N.; Wink, D. A.; Fukuto, J. M. *Free Radic. Biol. Med.* **2009**, *47*, 1130–1139.

(36) Dobmeier, K. P.; Riccio, D. A.; Schoenfisch, M. H. *Anal. Chem.* **2008**, *80*, 1247–1254.
(37) Heinecke, J. L.; Khin, C.; Pereira, J. C. M.; Suárez, S. A.; Iretskii, A. V; Doctorovich, F.; Ford, P. C. *J. Am. Chem. Soc.* **2013**, *135*, 4007–4017.
(38) Suárez, S. S. A.; Bikiel, D. D. E.; Wetzler, D. E. DE; Martí, M. A. M. A.; Doctorovich, F. *Anal. Chem.* **2013**, *85*, 10262−10269.
(39) Bagiyan, G. A.; Koroleva, I. K.; Soroka, N. V.; Ufimtsev, A. V. *Russ. Chem. Bull.* **2003**, *52* (5), 1135–1141.
(40) Wink, D. A.; Nims, R. W.; Darbyshire, J. F.; Christodoulou, D.; Hanbauer, I.; Cox, G. W.; Laval, F.; Laval, J.; Cook, J. A.; Krishna, M. C.; DeGraff, W. G.; Mitchell, J. B. *Chem. Res. Toxicol.* **1994**, *7* (4), 519–525.
(41) Singh, S. P.; Wishnok, J. S.; Keshive, M.; Deen, W. M.; Tannenbaum, S. R. *Proc. Natl. Acad. Sci. U. S. A.* **1996**, *93* (25), 14428–14433.
(42) Filipovic, M, In The Chemistry and Biology of Nitroxyl (HNO), Fabio Doctorovich, Patrick Farmer and Marcelo A. Marti; Elsevier Inc., 2017.
(43) Fukuto, J. M.; Cisneros, C. J.; Kinkade, R. L. *J. Inorg. Biochem.* **2013**, *118*, 201–208.
(44) Jocelyn, P. C. *Eur. J. Biochem.* **1967**, *2* (3), 327–331.
(45) Wrobel, A. T.; Johnstone, T. C.; Liang, A. D.; Lippard, S. J.; Rivera-fuentes, P. *J. Am. Chem. Soc.* **2014**, *136*, 4697–4705.
(46) Reisz, J. a; Zink, C. N.; King, S. B. *J. Am. Chem. Soc.* **2011**, *133* (30), 11675–11685.
(47) Cline, M. R.; Tu, C.; Silverman, D. N.; Toscano, J. P. *Free Radic. Biol. Med.* **2011**, *50* (10), 1274–1279.

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