Nitric Oxide Donors: Chemical Activities and Biological Applications

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I. Introduction

Although the use of glyceryl trinitrate (GTN) for medicinal purposes dates back to more than 150 years, little had been revealed about its physiological mechanism of action before the 1980s. It is well-

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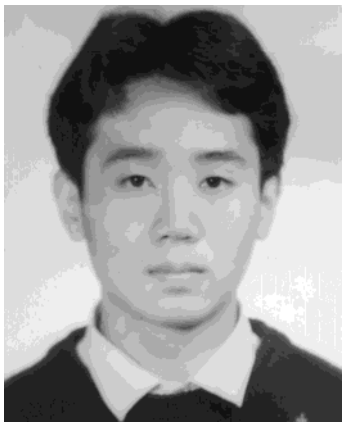
Professor Peng George Wang received his B.S. degree (1984) from Nankai University and his Ph.D. degree (1991) in Organic Chemistry from UC Berkeley. Then he moved to Scripps Research Institute as a postdoctoral fellow under Professor Chi-Huey Wong from 1992 to 1994. He started his independent academic career at the University of Miami in 1994 and then moved to Wayne State University in 1997, where he is a Professor in chemistry. His current interests lie in the areas of bioorganic and synthetic chemistry, especially the synthesis of complex carbohydrates and natural products, synthesis of natural and unnatural products through biosynthetic pathway engineering, biological chemistry of nitric oxide and reactive nitrogen and oxygen species; combinatorial chemistry; green chemistry; and design and synthesis of enzyme and receptor inhibitors.



Ming Xian was born in Leshan, China, in 1973. He earned his B.S. degree in Chemistry from Nankai University, China (1995). Then he continued his work as a joint-education graduate student between Nankai University (1995–1999) (with Professor Jin-Pei Cheng) and Wayne State University (1999–2003) (with Professor Peng George Wang). He received his Ph.D. degree from Nankai in 2000. He will obtain his Ph.D. degree from Wayne State in 2003. His doctoral research focuses on the synthesis of novel NO donors and carbohydrate natural products.

known that the epoch-making invention realized by Alfred Nobel in 1863 paved the way for controlled detonation of GTN. Therefore, when Nobel's physician recommended GTN as treatment of his angina pectoris, Nobel wrote "Isn't it the irony of fate that I have been prescribed N/G 1 [nitroglycerine] to be taken internally! They called it Trinitrin, so as not to scare the chemist and the public".[1] There would not be any irony for Nobel if he knew that it was nitric oxide (NO), released from GTN in vivo, that helps relieve angina. The surprising and exciting discovery of the multiple roles NO plays in physiological and pathophysiological functions in the humans earned Furchgott, Ignarro, and Murad the Nobel Prize in 1998.[2−4]

Xiaoping Tang received both B.S. and M.S. degrees in Chemistry from the University of Science and Technology of China. He obtained Ph.D. degree in Organic Chemistry from the University of Louisville, KY, in May 2000 under the guidance of Dr. K. Grant Taylor. After working as a postdoctoral fellow for Professor Peng Geroge Wang for about 1 year, he is currently a research scientist at CNH Technologies Inc. in Woburn, MA.

Xuejun Wu was born in 1973 in Luoding, a small town in south China. He entered Beijing Medical University in 1992 and received his early chemistry training at the National Research Laboratories of Natural and Biomimetic Drugs under the direction of Professor Lingtai Ma. In 1996, he obtained his B.S. degree and was admitted into Shanghai Institute of Organic Chemistry with exemption from graduate entrance exams. He completed his M.S. degree under the guidance of Professor Binghui Yang at State Key Laboratory of Bioorganic and Natural Products Chemistry. His research focused on the synthesis of nucleoside analogues with antitumor and antiviral activities. In 2000, he joined Professor Peng George Wang's group at Wayne State University as a research assistant and is involved in nitric oxide chemistry.

As a simple diatomic free radical, NO is generally considered to represent the biologically important form of the endothelium-derived relaxing factor (EDRF).[5] Cellular NO is almost exclusively generated via a five-electron oxidation of L-arginine, which is catalyzed by nitric oxide synthases (NOS).[6] Under physiological conditions, NO directly activates soluble guanylate cyclase (sGC) to transform guanosine triphosphate (GTP) into cyclic guanosine monophosphate (cGMP), followed by kinase-mediated signal transduction.[7] The endogenous formation of NO plays a key role in many bioregulatory systems including immune stimulation,[8] platelet inhibition,[9−13] neurotransmission,[14] and smooth muscle relaxation.[15−17]

NO is a colorless gas with a solubility of 2−3 mM in water. It may undergo chemical reactions with a variety of atoms and radicals.[18] For example, NO

Zhong Wen was born in Liaoning, China, in 1968. He studied chemistry at Nankai University, China, and received his Ph.D. degree in 2000 in Physical Organic Chemistry under the supervision of Professor Jin-Pei Cheng. In 2000 he joined the group of Professor Peng George Wang at Wayne State University as a postdoctoral fellow. His research interests include theoretical study of electronic structure and mechanism of nitric oxide (NO) donors and rational design of NO donors based on protein structures.

Tingwei Cai (born 1969) graduated with his B.S. (1992) and M.S. (1995) degrees from Beijing Medical University. He is presently a graduate student at Wayne Sate University. His doctoral research focuses on the synthesis of natural guanidine products and novel NO donors.

Adam Janczuk was born in Poland in 1976. After moving to the Detroit, MI, he received his B.S. degree in Chemistry in 1997 from Wayne State University. After a short stint in industry, he applied to Wayne State University's graduate program. Currently, he is near completing his Ph.D. studies under the guidance of Professor Peng G. Wang. His research interests include chemical and chemoenzymatic synthesis of carbohydrates, natural product synthesis, lanthanide-catalyzed reactions, and NO-related work.

may react readily with O_2 to give nitrogen dioxide (NO_2);[19] the reaction of NO with superoxide anion ($O_2^{\bullet-}$) generates peroxynitrite ($ONOO^-$), an oxidative species that is responsible for certain types of NO-mediated toxicity in vivo.[20] It can also interact with oxyhemoglobin to form methaemoglobin and nitrate.[21] Due to the instability and inconvenient handling of aqueous solutions of authentic NO, there is an increasing interest in using compounds capable of generating NO in situ, i.e., NO donors. Organic nitrates, such as GTN, may be the most well-known NO donors. In addition to organic nitrates, many other chemicals can be transformed into NO in vitro or in vivo. Due to the diversity of NO donor structures, the pathway for each class of compounds to generate NO could differ significantly, e.g., enzymatic vs nonenzymatic, reductive vs oxidative, etc. As each class of compound offers distinct biochemical properties, it allows one to choose a compound that best meets the demands of specific investigations.

Several reviews on the biology of NO have been published over the past 10 years.[21−26] The pharmaceutical aspects of NO donors have also been extensively reviewed by several authors.[27−31] In this review, our object is to summarize the syntheses, chemical/enzymatic mechanisms of NO release, biomedical applications, and current trends on the development of NO donor compounds.

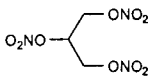
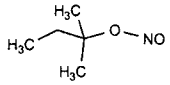
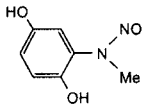
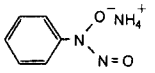
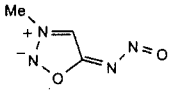
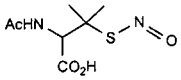
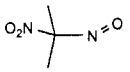
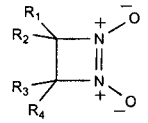
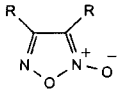
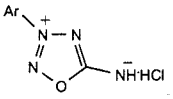
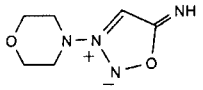
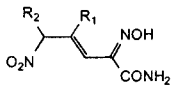
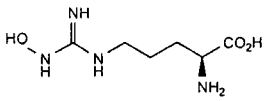
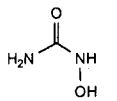
II. Current Major Classes of Nitric Oxide Donors

All nitrogen−oxygen-bonded compounds have the potential to decompose, be oxidized, or be reduced to produce reactive nitrogen species. Major classes of current NO donors (except diazeniumdiolates, which will be reviewed by Hrabie and Keefer) along with their individual pathway of NO generation are summarized in Table 1. The structural dissimilarities of the diverse NO donors have led to remarkably varied chemical reactivities and NO-release kinetics. A number of cases of NO generation can be induced by thiols, basing on either reduction or transnitrosation. In fact, thiols represent a main cofactor for NO releasing of furoxans, organic nitrite, nitrate, and other nitro compounds. Many NO donors are so heat/light sensitive that they can decompose spontaneously if precautionary measures are not taken. Some compounds are very hygroscopic and may be pH-dependent. Ligands can even induce and facilitate the NO releasing from some metal−NO complexes. On the contrary, some NO donors are relatively stable and cannot generate NO without the presence of oxidants or metal-ion catalysis. In this section we will discuss each class of NO donors, focusing on their syntheses, reactions, NO-releasing mechanisms, and biological applications.

A. Organic Nitrates

Organic nitrates ($RONO_2$) are nitric acid esters of mono- and polyhydric alcohols, representing the oldest class of NO donors that have been clinically applied. Representative organic nitrates include glyceryl trinitrate (GTN), pentaerythrityl tetranitrate

Table 1. Current Major Classes of NO Donors[a]

Index, Name	Representative Compounds	Pathway of NO Generation	
		Non-enzymatic	*Enzymatic*
A. Organic nitrates		thiols	Cyt-P450, GST and a membrane-bound enzyme
B. Organic nitrites		hydrolysis and trans-nitrosation; thiols; light; heat	cytosolic and microsomal enzymes; xanthine oxidase
C. Metal-NO complexes	$Na_2[Fe(CN)_5(NO)] \cdot 2H_2O$	light; thiols; reductants; nucleophiles	a membrane-bound enzyme
D. *N*-Nitrosamines		·OH; light	Cyt-P450 related enzymes
E. *N*-Hydroxyl nitrosamines		light; heat	peroxidases
F. Nitrosimines		thiols; light	?
G. Nitrosothiols1		spontaneous; enhanced by thiols, light and metal ions	unknown enzymes
H. *C*-nitroso compounds		light; heat	?
I. Diazetine dioxides		spontaneous; thiols	?
J. Furoxans and benzofuroxans		thiols	unknown enzyme
K. Oxatriazole-5-imines		thiols	?
L. Sydnonimines		spontaneous, enhanced by light, oxidants and pH>5	prodrugs require enzymatic hydrolysis
M. Oximes		spontaneous; O_2/Fe^{III}-porphyrin	?
N. Hydroxylamines		autoxidation enhanced by metal ions	catalase/H_2O_2
O. *N*-Hydroxyguanidines		oxidants	NOSs, Cyt-P450
P. Hydroxyureas		H_2O_2/CuZn-SOD or ceruloplasimin; H_2O_2/Cu^{2+}; heme-containing proteins	peroxidase

[a] The representative may differ greatly to individual NO donor of the corresponding class. Please refer to the text and references therein for details.

(PETN), isosorbide dinitrate (ISDN), isosorbide 5-mononitrate (ISMO), and nicorandil (Figure 1). The partially denitrated metabolites of GTN, glyceryl dinitrates (GDN) and mononitrates (GMN), are still pharmacologically active but considerably less potent than GTN.

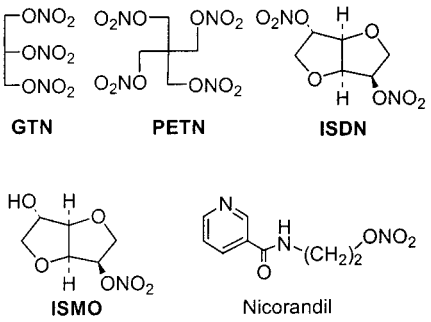


Figure 1.

1. Synthesis

In general, organic nitrates can be readily prepared from the esterification of corresponding alcohols or the substitution between reactive alkyl halides and $AgNO_3$ (Scheme 1).[32]

Scheme 1

$$R{-}OH + HNO_3 \xrightarrow{H_2SO_4} R{-}ONO_2 + H_2O$$

$$R{-}X + AgNO_3 \longrightarrow R{-}ONO_2 + AgX \quad (X = Cl, Br)$$

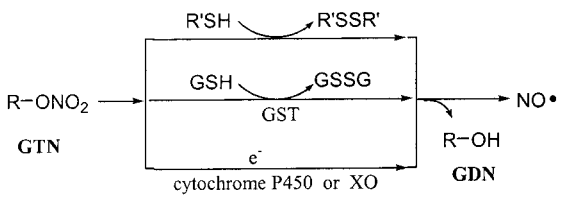
2. Physical Properties

Most organic nitrates are only sparingly soluble in water. When properly sealed and protected from light, their solutions can be kept in ethanol or DMSO for months to years. Organic nitrates are generally stable in neutral or weakly acidic aqueous solution.[33] The gas-phase homolytic bond dissociation energy of the $O{-}NO_2$ bond in aliphatic nitrates is ca. 41 kcal/mol.[34] Under strong alkaline conditions, they are susceptible to hydrolysis (S_N2 nucleophilic substitution to give alcohol and nitrate), β-H elimination (forming alkene), and α-H elimination (producing aldehyde and nitrite).[35,36]

3. Biotransformation.

The major biological effects of nitrates are attributable to the formation of NO. The NO release from organic nitrates requires either enzymatic or nonenzymatic bioactivation where a three-electron reduction is involved.[37,38] When GTN is exposed to mammalian tissues, NO is released generally from the C-3 carbon.[38,39] Although the biochemical process of NO release from GTN has not been fully defined, it is likely that multiple intracellular and extracellular pathways (Scheme 2) contribute to NO formation

Scheme 2



from these compounds in vivo.[40−42]

It has been suggested that cellular thiols are involved in nonenzymatic formation of NO from GTN.[43−46] Most studies showed that manipulation of thiols either in vivo or in vitro influences the response to GTN.[47−49] In this nonenzymatic process, sulfhydryl groups donate reducing equivalents to form their respective disulfides (RSSR); NO_2^- ion is released as the major nitrogenous metabolite.[43−46,50] Virtually all thiol compounds decompose organic nitrates to NO_2^-, whereas only cysteine, *N*-Ac-cysteine, and thiosalicylic acid (but not glutathione) promote concomitant generation of NO free radical.[43−46,51,52] It was once proposed that thionitrate ($RSNO_2$) was formed as an intermediate via transesterification between the nitrate ester and the thiol.[53] Recently, Zavorin et al. suggested that the thiol-mediated NO release from nitrate esters was due to an initial two-electron reduction followed by a thiolate nucleophilic attack on the nitrate group, via a sulfenate intermediate (rather than a thionitrate).[54]

Since the degradation of GTN by thiols is very slow at room temperature, it appears that the release of NO from reasonable concentrations of GTN and thiols almost certainly involves an enzymatic process.[55] The site of enzymatic biotransformation of organic nitrates has not yet been identified. Both an NADPH-dependent cytochrome P450 pathway[56−58] and certain isoenzymes of the glutathione-*S*-transferase (GST) family[59] were believed to be involved in the bioactivation of organic nitrates.[60−64] The cytosolic GSTs catalyze the attachment of GSH to one of the nitrate groups of GTN, giving rise to an unstable thionitrate ($RSNO_2$) which then reacts with a second GSH molecule to yield GDN, nitrogen oxide, and GSSG.[65−69] Alternatively, GTN can react with the ferrous−heme moieties of haemoglobin and myoglobin to give GDN and NO. It is suggested that GTN may be reduced by the Fe(II) active site in the presence of NADPH.[58,70] Millar et al. observed that the reduction of GTN to NO could be catalyzed by xanthine oxidase (XO) in the presence of NADH.[71]

Recently, Wong and Fukuto proposed that the bioactivation of nitrate esters involved reduction to organic nitrites in the presence of a reduced flavin species (FMN/NADH), followed by the conversion to a nitrosothiol (e.g., GSNO). NO is released via a variety of pathways including reduction by FMN/NADH.[72] Booth et al. suggested that, in addition to the formation of NO, GTN might generate a nitroxy anion (NO^-) which releases a calcitonin gene-related peptide (CGRP, a small neuropeptide that is capable of potent vasodilation).[73]

4. Nitrate Tolerance

In general, the response to these organic nitrates is markedly attenuated after prolonged or repeated exposure, known as "nitrate tolerance". The mechanisms of nitrate tolerance are unclear. It was once ascribed to the depletion of the sulfhydryl groups,[74] down-regulation of enzymes involved in bioactivation,[75−78] desensitization of the target enzyme guanylase cyclase, or a decrease in GTN biotransformation.[79,80] Now it appears unlikely that GTN tolerance is mediated by thiol depletion.[81−83] Physiological mechanisms, such as changes in plasma neurohormonal levels and receptor density, may also contribute to nitrate tolerance.[84]

Recently, it was suggested that GTN administration induced an increase in vascular production of superoxide ($O_2^{\bullet-}$) that could chemically inactivate NO derived from GTN, resulting in blunting of the sensitivity of the vascular tissue of vasoconstrictors by reduction of superoxides.[85,86] Several studies have indeed indicated that the sensitivity of the vasculature to organic nitrates might at least in part be preserved by the concomitant treatment with ascorbic acid,[87,88] vitamin E,[89] or other antioxidants.[90] The formation of superoxide induced by PETN is much lower than by GTN both in vitro and in vivo.[91,92] To date, PETN is the only nitrate on the market reported to be poor or free of nitrate tolerance,[93] and several compounds that are derived from PETN have been patented.[94]

5. Biological Applications

Organic nitrates have long been used to relieve angina pectoris, a disease state caused by constriction of heart arteries. In fact, GTN has a relaxant effect on all types of vessels. Coronary arteries have been found to be more sensitive to GTN than peripheral arteries. GTN has also been used as treatment of acute myocardial infarction,[95] congestive heart failure,[96] as well as blood pressure control. In addition to relaxation of vascular smooth vessels, GTN is successful in the treatment of children with anal fissures when administered as an ointment[97] and as an alternative to sildenalfil (Viagra) when topically used as a spray to the shaft of the penis.[98] Rectal administration of GTN has been claimed as treatment and prophylaxis of inflammatory bowel disorders.[99] ISDN has been suggested as a long-term transdermal therapy in preeclamptic women to avoid maternal hypertension and fetal distress.[100] It was recently reported that organic nitrates eventually inhibited the proliferation of smooth muscle cells (SMC), which was associated with the pathogenesis and progression of atherosclerosis.[101−103]

The requirement for specific thiols and/or enzymatic bioactivation for NO release renders organic nitrates less ideal compounds for the generation of predictable rates of NO in vitro. However, organic nitrates possess a clear advantage over other NO donor classes for use in animal studies since most of their in vivo effects are reasonably well documented.

B. Organic Nitrite

Organic nitrites are esters between alcohols and nitrous acid. Some of them, such as butyl nitrite (BN), isobutyl nitrite (ISBN), *tert*-butyl nitrite (TBN), amyl nitrite (AMN), and isoamyl nitrite (IAMN) (Figure 2), have been clinically used as vasodilators for a long time.

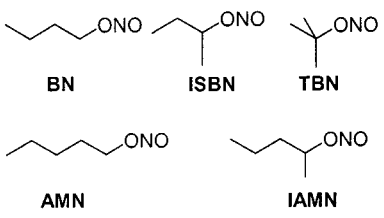
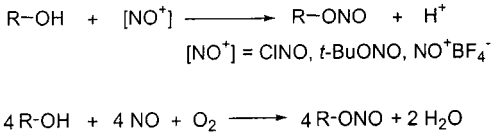


Figure 2.

1. Synthesis

Organic nitrites can be prepared by reacting alcohols with nitrous acid or other nitrosating agents such as nitrosyl chloride (ClNO) and nitrosonium salts ($NO^+BF_4^-$, $NO^+ClO_4^-$).[104] Transesterification between *tert*-butyl nitrite and other alcohols can also be used to synthesize nitrites.[105] Recently, it was reported that alkyl nitrites could be synthesized from the reaction of alcohols with gaseous NO in the presence of air, where nitrous anhydride, generated in situ, likely serves as the nitrosating agent.[106] The general synthetic procedures are shown in Scheme 3.

Scheme 3



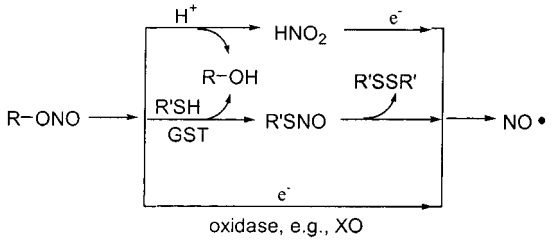
2. Physical Properties

In general, the boiling points of alkyl nitrites are significantly lower than those of the corresponding alcohols. Due to the high electronegativity of oxygen, nitrosyl nitrogen atoms exhibit high electron deficiency, rendering them highly susceptible to nucleophilic attack by oxygen-, nitrogen-, and sulfur-nucleophiles, leading to transfer of a nitrosyl group (transnitrosation).[104,107] The O−NO homolytic bond dissociation energies of alkyl nitrites are 36−41 kcal/mol.[108] The photochemical and thermochemical decomposition of nitrites first leads to homolysis of the O−NO bond, followed by either H-abstraction (for straight-chain nitrites)[109−112] or ring opening (for cyclic nitrites),[113,114] giving rise to a 1,5-rearrangement product. Photolysis of *tert*-butyl nitrite produces nitrosomethane and acetone, via a *tert*-butyloxy radical as an intermediate.[115]

3. Reactions

Organic nitrites can generate NO in vivo.[116] The transformation of nitrites to NO requires one-electron reduction. They also undergo rapid hydrolysis to give nitrite ion and the corresponding alcohol. The nitrite ion is not an active intermediate in vascular metabolism of nitrite esters but can be reduced to NO.

Scheme 4



Since the nitrosyl moiety of nitrites can be readily transferred to a sulfhydryl group,[117,118] *S*-nitrosothiols may be formed from organic nitrites in vivo. Kowaluk and Fung[119] showed that NO release from nitrites, an enzymatic process in vascular smooth muscle, was

primarily associated with the cytosolic (as opposed to a microsomal fraction in the case of the organic nitrates[120]). Meyer et al. found that cytosolic GSTs could catalyze the reaction of alkyl nitrites with GSH to produce GSNO.[121] Ji et al. also observed that microsomal GST catalyzed the formation of *S*-nitrosothiols such as GSNO, playing a significant role in the metabolism of alkyl nitrites in biological membranes.[122] On the other hand, Doel et al. showed that xanthine oxidase XO could catalyze the reduction of organic nitrites to NO under anaerobic conditions.[123]

4. Biological Applications

Alkyl nitrites used as inhalants cause vasodilation, increased heart rate, and decreased systolic blood pressure.[124] The vasodilatory effect following inhalation of organic nitrites has been used to relieve angina pectoris since 1867.[125] They are more potent than nitrate esters. Amyl nitrite and *n*-butyl nitrite have been commonly used in relieving angina pectoris. Unlike nitrates such as GTN, constant infusion of organic nitrites in the same animal model does not produce tolerance.[126−128] In fact, Fung and Bauer claimed the long-term, continuous administration of organic nitrites as an effective vasodilator therapy.[129,130]

C. Metal–NO Complexes

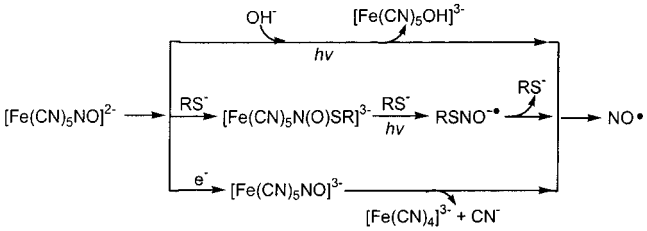
NO is a powerful ligand to metal ions, with binding constants often much higher than those of CO and O_2, and dominates the coordination sphere of the metal.[131,132] In fact, the principal targets for NO under bioregulatory conditions are metal centers (primarily iron).[133,134] Conversely, the metal nitrosyl compounds (M−NO) may be NO donors.[135] The most widely studied metal nitrosyl is sodium nitroprusside ($Na_2[Fe(CN)_5NO]$, SNP). Recently, the potential of iron−sulfur cluster nitrosyls and other metal nitrosyl compounds (M−NO, M = Ru, Cr, Mo, etc.) as NO donors have received much attention.

The general features and syntheses of metal nitrosyl complexes have been well documented by Richter-Addo and Legzdins.[132] One feature of metal nitrosyls is the variable nature of the NO ligand, on going from $M{\cdot}NO^-$ to $M{\cdot}NO^+$ with increasing oxidation state of metal. This nature, as indicated by the IR spectrum of NO functionality ($\nu_{N=O}$), in turn largely determines the reactivity of NO. For example, the nitrosyl moiety in the nitroprusside anion (Fe(III)·NO complex) exhibits significant NO^+ character and thus is subject to attack from a variety of nucleophiles. In contrast, Severina et al.[136] found no hypertensive effect for metal nitrosyls ($[M(CN)_5NO]^{n-}$) where the ligand NO was either neutral (M = Co) or anionic (M = Cr). Moreover, NO is such a strong a ligand that it exhibits a significant *trans*-effect, i.e., it disfavors the coordination at its *trans*-position or, conversely, is destabilized by strong ligands at *trans*-position (*trans*-labilization).

1. Sodium Nitroprusside (SNP)

For over 70 years, SNP has been used clinically to reduce blood pressure, e.g., in hypertensive emergencies. The vasodilation effect of SNP is through the formation of nitric oxide.[137] SNP in crystalline form ($Na_2[Fe(CN)_5NO]{\cdot}2H_2O$) can be stored for years at room temperature when kept dry and protected from light. SNP solution is extremely photosensitive,[138] and its decomposition is accelerated by oxygen.[139] Although the mechanism of NO release from SNP is not fully understood, it is clear that NO release requires either irradiation with light or one-electron reduction and is usually enhanced by thiols (Scheme 5).

Scheme 5



In biological systems, NO release from SNP may occur both enzymatically and nonenzymatically, requiring the presence of vascular tissue or catalyzed by reducing agents.[140] Under physiological conditions, NO release by photolytic production is not significant.[141] Nonenzymatic one-electron reduction by reducing agents (e.g., thiols, hemoproteins, and possibly ascorbate) that are abundant in most biological tissues does release significant amounts of NO. It is likely that vascular tissue, including cell membranes and smooth muscle cell membranes, serves this reducing function. A membrane-bound enzyme may be involved in the generation of NO from SNP in biological tissues, and either NADH or NADPH appears to be required as the cofactor.[140,142−144]

Attack of thiolate anions (membrane-bound or free) on $[Fe(CN)_5NO]^{2-}$ may lead to the formation of NO in a multisequence manner: (i) the very fast reversible formation of the red intermediate $[Fe(CN)_5N(O)SR]^{3-}$,[145] (ii) its subsequent photooxidation−substitution to generate *S*-nitrosothiol radical ($RSNO^{\bullet-}$) under lower energy irradiation,[146] and (iii) secondary processes giving rise to different iron complexes, disulfides, and NO species. Upon addition of SNP to tissues, formation of nitrosyl−iron complexes with thiols has been detected, which might be in dynamic equilibrium with *S*-nitrosothiols and dinitrosyl−iron complexes.[147,148]

Since the reduction and subsequent decomposition of SNP is accompanied by cyanide release (a maximum of 5 equiv of CN^- per mole SNP), this leads to pronounced cellular toxicity.[149] It limits the use of SNP as NO donor for in vivo studies. Moreover, through spontaneous release of NO, SNP is involved in the formation of cytotoxic peroxynitrite.[150] Reports also indicate that SNP increases the toxicity of H_2O_2.[151]

Nevertheless, NO is such a powerful vasodilator that SNP is effective at doses that do not produce toxic amounts of cyanide. It can be rapidly degraded after cessation of infusion, and the byproduct cyanide is rapidly metabolized. SNP is clinically used for treatment of advanced heart failure and hyperten-

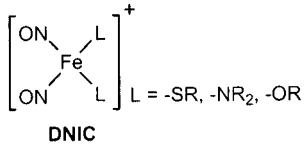


Figure 3.

sion. Furthermore, Zhang et al.[152,153] showed that SNP significantly reduced the cerebral infarct size. In the presence of vascular tissue, SNP also inhibits platelet aggregation.[154] Recently, Bivalacqua et al.[155] reported that transurethral administration of SNP could induce an erectile response in cats with minimal side effects.

2. Dinitrosyl Iron Thiol Complexes (DNICs)

DNICs (Figure 3), a type of mononuclear iron nitrosyls formed by constitutive iron and NO, were discovered in biological tissues.[156−158] They contain a range of O/N/S/P ligands.[159−165] The most widely studied DNICs are the complexes of L-cysteine and GSH, with a molar ratio of Fe^{2+}/RSH of 1:20 or 1:2. Their solutions can be readily prepared from the aqueous solutions of ferrous sulfate, L-cysteine or GSH, and NO under inert atmosphere.[159,166−168]

The stock solution of DNIC can be stored at −80 °C for weeks without noticeable decomposition. The aqueous solution of DNIC is characterized by its broad UV−vis absorption around 320 nm. Both paramagnetic and diamagnetic forms exist in DNIC, and the former can be monitored by EPR. At room temperature, low-mass DNICs are quite unstable and readily decompose into NO. Kinetics and yields of NO release from DNICs are subject to the concentration of the thiols.[169] Acids accelerate the transformation of DNIC into RSNO and NO.[170] By ligand exchange, paramagnetic protein-bound DNICs can be instantly formed from low-mass DNIC. These protein-bound DNICs are fairly stable and may serve as a reservoir for low-mass DNICs.

DNICs have been suggested as storage and transporters of NO in vivo[167,171,172] as well as intermediates of iron-catalyzed degradation and formation of *S*-nitrosothiols.[173] They have been shown to inhibit platelet aggregation, relax vascular vessels, reduce blood pressure, enhance cardiac resistance to ischemia, and reperfusion.[174−177] They also exhibit inhibition to glutathione reductase and GST[178,179] and induce accumulation of heat shock protein HSP70.[180,181] Recently, it was suggested that ion channel activity could be modulated through transfer of dinitrosyl iron moiety from DNICs.[182]

3. Iron–Sulfur Cluster Nitrosyls

Iron−sulfur [Fe−S] clusters exist as an integral part of several enzymes (Figure 4).[183] The interaction of NO with these natural structures gives rise to iron−sulfur cluster nitrosyl complexes. Synthetic iron−sulfur cluster nitrosyls include Roussin's black salt (RBS, $[Fe_4S_3(NO)_7]^-$), Roussin's red salt (RRS, $[Fe_2S_2(NO)_4]^{2-}$), Roussin's red "esters" ($Fe_2(SR)_2(NO)_4$, RRE, R = aliphatic group), and tetranitrosyl-tetra-μ3-sulfidotetrahedro-tetrairon ($(FeNOS)_4$). The methyl ester of RRE has been found in natural vegetable

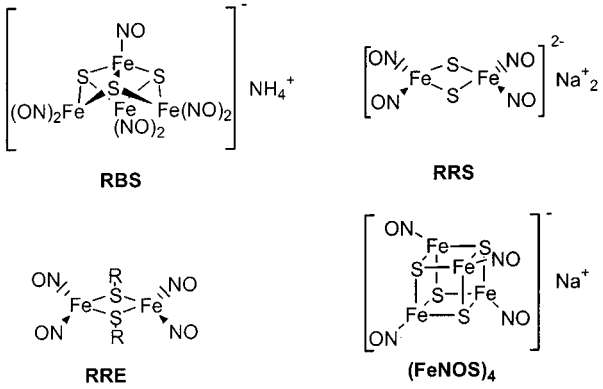
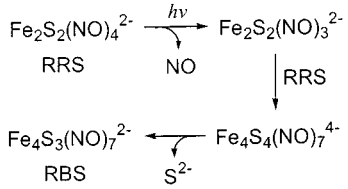


Figure 4.

sources.[184] The syntheses of RBS,[185] RRS,[185] RRE,[186−188] and $(FeNOS)_4$[189,190] have been reported. Recently, water-soluble RRE (R = $CH_2CH_2SO_3^-$) was synthesized.[191]

Photolysis of RRS in aerobic aqueous solution leads to quantitative formation of RBS and NO (Scheme 6), and the less photoreactive RBS further undergoes

Scheme 6



photodecomposition to generate NO and ferric precipitates.[192] Recently, it has been determined that the molar ratios of NO produced per Fe−NO complex were 0.50 for RRS and 5.9 for RBS.[193] Under photolysis, 1 mol of RRE (R = $CH_2CH_2SO_3^-$) in aerated solutions produces 3.8 mol of NO.

Flitney et al. tested RBS as an NO-releasing drug for thermo-/photochemical delivery of NO to vascular and brain tissues.[194,195] Matthew et al. observed that sequestered or bound RBS, upon photoactivation, liberated NO by a process that could be controlled by the wavelength, intensity, and duration of the incident light.[196] Butler and co-workers reported that RBS and $(FeNOS)_4$ dilate precontracted, internally perfused rat tail arteries and the patterns of vasodilator responses was dose-dependent, following an NO-mediated mechanism.[194] The apparent ease with which both clusters were able to penetrate the endothelial cell membrane is probably related to their high solubility in nonpolar solvents, which allowed RBS to be rapidly taken into the endothelial cells and slowly release NO for hours.[194] Ludbrook et al. reported that RBS inhibited the ADP-induced platelet aggregation by extracellular release of NO.[197] Payne et al. suggested that the bacteriostatic effect of RBS, RRE, and $(FeNOS)_4$ could be attributed to the interaction between iron−sulfur proteins and NO.[198]

4. Ruthenium Nitrosyls

On the basis of the well-known affinity of ruthenium centers for NO,[199] ruthenium nitrosyls (Figure 5) have drawn considerable attention as photosensi-

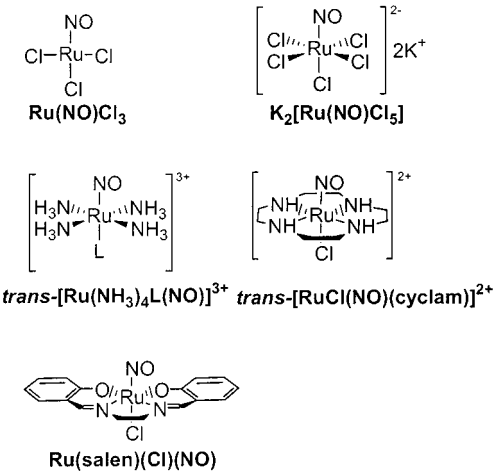
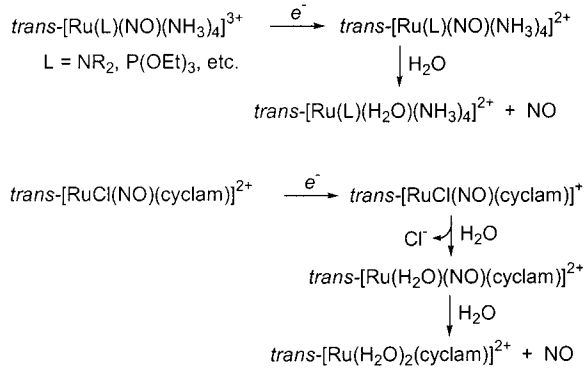


Figure 5.

tive precursors of NO.[200−207] By varying the π-bonding of other ligands and the *trans* effect, the affinities of ruthenium ion for NO and the reduction potential of the complex can be modulated so as to tune the dissociation rates of the Ru−NO bond.[208,209]

Clarke and Gaul showed that, by altering the ligand L, NO could be released from *trans*-[NO(L)-$(NH_3)_4$Ru]Cl_3 upon reduction (Scheme 7).[208] The

Scheme 7



bound NO exhibits strong nitrosonium (NO^+) character in *trans*-[NO(L)$(NH_3)_4$Ru]$^{3+}$ (L = imidazole, pyridine, sulfite, etc.), which releases NO upon irradiation.[210,211] Lopes et al. found that, in *trans*-[NO-(L)$(NH_3)_4$Ru]Cl_3 (L = imidazole), the imidazole ligand was coordinated to the Ru(II) through a carbon atom which facilitates NO release in the reduced form.[212] Recently, Lang et al. reported that the reduction of *trans*-[RuCl(NO)(cyclam)]$^{2+}$ (cyclam = 1,4,8,11-tetraazacyclotetradecane) led to slow NO release, suggesting that this type of compound could be used as long-lasting vasodilator.[213] Interestingly, together with a biologically accessible reduction potential, the strong *trans*-labilization effect of phosphito ligand facilitates the NO release from *trans*-[NO(P(OEt)$_3$)-$(NH_3)_4$Ru]$^{3+}$ after reduction.[214,215]

5. Iron–Porphyrin Nitrosyls

Soluble guanylyl cyclase (sGC) contains a five-coordinated ferrous heme with a histidine as the axial ligand at the fifth coordinating position.[216] During the activation of sGC, NO binds to the sixth coordination position of the heme iron and leads to the breakage of the histidine-to-iron bond, yielding a five-coordinated nitrosyl−heme complex. It is believed that this cleavage of the histidine-to-iron bond initiates a conformational change that results in the activation of sGC (Figure 6). The stimulation of sGC without

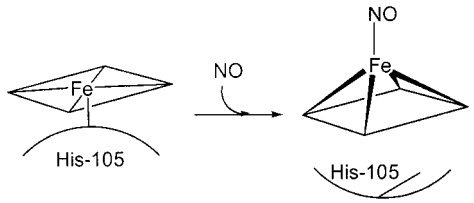
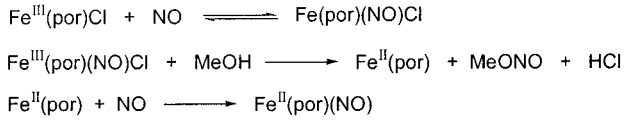


Figure 6.

the presence of NO by protoporphyrin IX, the iron-free precursor of heme, has supported this proposed activation mechanism.[217]

Nowadays, nitrosyl hemoglobin (HbNO) has been intensively studied and it is evident that ferrous complexes of both natural and synthetic porphyrins generally have extremely high affinities for NO. In connection with the studies of fundamental chemistry of heme NO adducts, Bohle et al. investigated a class of ferrous nitrosyl complexes with synthetic tetraarylporphyrins.[218] These Fe(porphyrin)(NO) complexes are readily prepared by reductive nitrosylation of the corresponding Fe(porphyrin)Cl complexes with NO in the presence of excess methanol and 2,6-lutidine (Scheme 8).[219] Their research results sug-

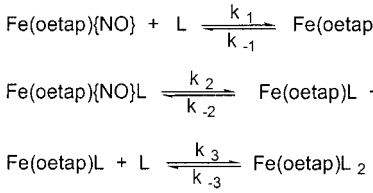
Scheme 8



gested that a variety of factors can lead to a dramatic variation by 6 orders of magnitude in the rate of NO dissociation from Fe(porphyrin)(NO) when treated with coordinating ligands.

They extended their studies to include soluble iron (II) octaethyltetraazaporphyrin, [Fe(oetap)]. The iron-(II) nitrosyl complexes [Fe(oetap)(NO)] can be prepared by the similar method mentioned above. In contrast to the nitrosyl derivative of iron(II) octaethylporphyrin [Fe(oep)(NO)], [Fe(oetap)(NO)] exhibits fast ligand-promoted NO dissociation in the presence of coordinating ligands such as pyridine and *N*-methylimidazole.[220] The denitrosylation kinetic research of [Fe(oetap)(NO)] suggests a mechanism which involves rapid equilibrium binding of axial ligand followed by a rate-determining loss of NO from the six-coordinate intermediate (Scheme 9).

Scheme 9



The delicacy of the biological system is amazing if you look into the blood-sucking mode of the small insect *Rhodnius prolixus*, though you may not like it. Like most blood-sucking insects, *Rhodnius* secrets a saliva rich in proteins that facilitate feeding by interfering with hemostasis in the mammalian host. Four of these salivary proteins termed nitrophorins (NP1−NP4) are heme-containing. They store and transport NO and also bind with histamine. NP2 even interferes with the blood coagulation cascade at the factor X maturation.[221] Data from Kaneko et al. suggested that NP2 was tightly adhesive to the membranes to transport NO into the cell during the insect sting.[222] Nitrophorins utilize a ferric heme for binding NO or histamine. Binding of NO induces a conformational change that leads to ejection of three water molecules from the spacious distal pocket, and then the key loops collapse into and completely fill the distal pocket. This amazing procedure sequesters the NO−heme complex and prevents reaction with solvent molecules such as water, oxygen, hydroxide, and thiols. Binding of NO is stronger at the pH of salivary gland (approximately 5) than at the pH of host tissue (approximately 7.5), and release of NO is therefore facilitated upon delivery to the host. Ferrous (FeII) heme, for example, the trigger structure of sGC, binds NO about 6 orders of magnitude more tightly than ferric (FeIII) heme,[223] and this indicates that the stabilization of ferric heme in nitrophorin also determines the release of NO. In *Rhodnius*, binding of histamine occurs in the same place that binds NO, and this binding not only contests against the immune response of host, but also serves to make the NO release irreversible so that it can result in more efficient vasodilatory activity.[224]

D. *N*-Nitrosamines

N-Nitrosamines were first reported by Geuther in 1863.[225] They were used occasionally as solvents or synthetic intermediates in the preparation of hydrazines and diazoalkanes.[226] In general, *N*-nitroso compounds can be divided into two different types (Figure 7). Type I includes dialkyl, alkyl aryl, and diaryl nitrosamines. Type II compounds are nitrosamines with an electron-withdrawing group attached to the nitrogen bearing the NO group, e.g., *N*-nitrosamides, *N*-nitrosoureas, *N*-nitrosoguanidines, *N*-nitrosocarbamates, and other *N*-acyl-*N*-nitroso compounds. In 1956, Magee and Barnes reported that nitrosodimethylamine induced liver cancer when fed to rats.[227] Later it was found that nitrosamines could alkylate proteins and nucleic acids.[228,229] As a result of these early findings, *N*-nitrosamines are generally considered as carcinogens.[230] However, some reports show that *N*-nitrosamines have vasorelaxant activity and appear to stimulate sGCs.[231,232] Since their nitroso group could be homolytically or heterolytically transferred to another species, these *N*-nitroso compounds are considered a class of NO donors.

1. Synthesis

Endogenous nitrosamines are formed by nitrosation of amines in the body, via acid- or bacterial-catalyzed reaction, with nitrite or oxidative products

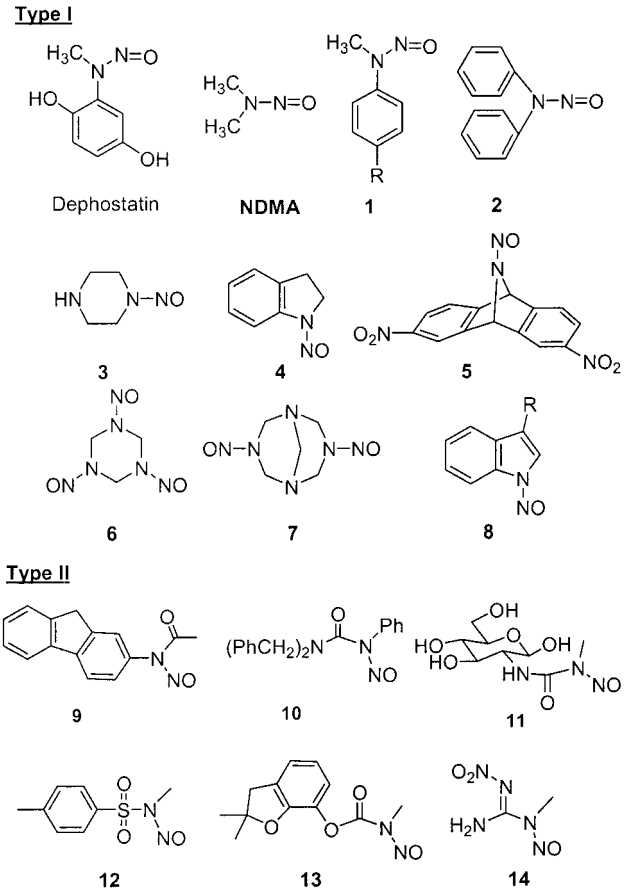
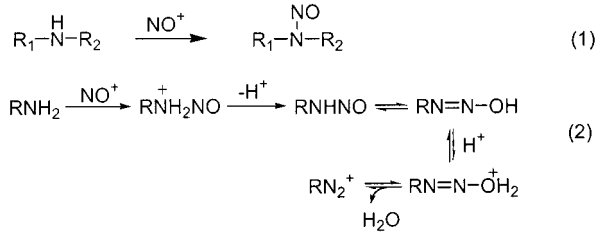


Figure 7.

of NO generated during inflammation or infection.[233,234] Exogenous nitrosamines are synthesized by the reaction of nitrosating agents with the corresponding amines (Scheme 10).[235] The nitrosating

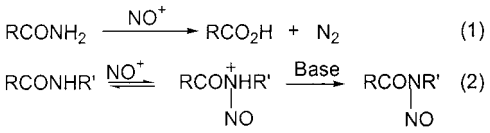
Scheme 10



agent, a source of the nitrosonium ion (NO^+), can be either nitrous acid (HNO_2), nitrosyl chloride (NOCl), dinitrogen trioxide (N_2O_3), dinitrogen tetraoxide (N_2O_4), nitrosonium tetrafluoroborate ($NO^+BF_4^-$), or alkyl nitrites.[236,237] Primary amines react readily with nitrosating agents to give products of deamination. The intermediates, primary nitrosamines (RNHNO), are not stable; therefore, after a series of subsequent reactions, they give rise to diazonium ions (RN_2^+) which then decompose to the final products. The reactions of secondary amines can stop at the nitrosamine stage, since there are no α-hydrogen atoms available for the necessary proton-transfer reactions leading to diazonium ion formation.

Nitrosation of primary amides results in deamination, producing carboxylic acid and nitrogen as products. Secondary amides, when nitrosated, give

Scheme 11



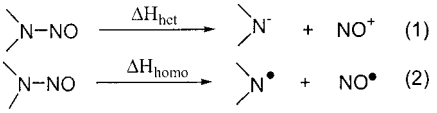
the corresponding nitrosamides in a reversible process. To obtain good yields of the nitrosamides, bases are often added to remove the acid formed (Scheme 11). This reaction also occurs with ureas and carbamates.

2. Physical Properties and Reactions

Most *N*-nitrosoamines are liquids with limited water solubility, except for those of three carbon atoms or less. These compounds are relatively stable in aqueous solution at physiological pH but are light sensitive. They are soluble in common organic solvents. All *N*-nitroso compounds show ultraviolet absorption in the 230−240 and 330−350 nm region. In the infrared region *N*-nitroso compounds exhibit a characteristic but weak band at 1445−1490 cm^{-1} (N═O stretching), which is different from the analogous band at 1605−1620 cm^{-1} in *C*-nitroso compounds and nitrite esters.[238,239]

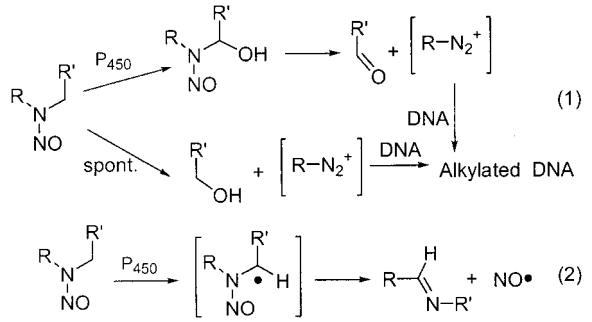
N-Nitrosamines are potential $NO^{\bullet}/NO^{+}$ donors through homolytic or heterolytic cleavage of the N−NO bond (Scheme 12). Their NO-releasing potential can be evaluated in terms of N−NO bond energy. Studies have shown that the homolytic cleavage of N−NO bonds, generating NO radical, is thermodynamically more favorable than the heterolytic cleavage, which generates NO^{+}, by 23−45 kcal/mol.[240,241]

Scheme 12



The established mechanism for carcinogenesis by *N*-nitrosamines is α-hydroxylation catalyzed by a variety of oxidases and oxygenases (such as cytochrome P450-related enzymes). Decomposition of α-hydroxy-*N*-nitroso compounds produces powerful alkylating agents which damage DNA. *N*-Nitrosamines also have the potential to decompose spontaneously in vivo leading to the formation alkylating agents as well (Scheme 13, eq 1). Aliphatic *N*-nitroso compounds such as *N*-nitrosodimethylamine (NDMA) produce little NO; this in part may be due to their relatively high N−NO bond dissociation energies (BDEs); therefore, only dealkylation is preferred.[241] Another event, denaturization, which accounts for 10−20% of total nitrosamine metabolism, is carried out by the same cytochrome P450-related enzyme. After one-electron oxidation of the α-carbon atom, a highly unstable α-nitro amino radical is generated, which readily releases NO (Scheme 13, eq 2).[242] For example, compounds **2** and **3** release NO in the liver and blood with yields of 1% and 0.01%, respectively.[243] The nitrosation products of hexamethylenetetramine, 1,3,5-trinitrosoheuahydro-1,3,5-triazine (**6**) and 3,7-dinitroso-1,3,5,7-tetrazabicyclo[3.3.1]nonane (**7**), have been shown to form NO at yields of 3.1% or 1.3% in vitro at 37 °C (1 h, pH 7.4), respectively.[244]

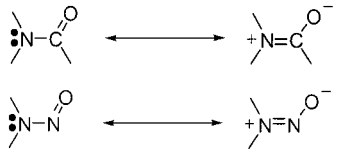
Scheme 13



N-Aryl-*N*-nitrosamines have higher NO-releasing potentials than *N*-alkyl-*N*-nitrosamines, because the resonance effect between the aromatic ring and neighboring nitrogen increases the NO-generating ability. Electron-withdrawing groups on the aromatic ring can weaken the N−NO bond and enhance the NO releasing ability. Some derivatives of **2** generate NO spontaneously and are therefore good nitrosating agents.[245] When the resonance effect is weakened by *ortho*-substituted groups or *N*-nitrosamines have a bulky *N*-substituted group such as *tert*-butyl, their NO-releasing ability diminishes.[246]

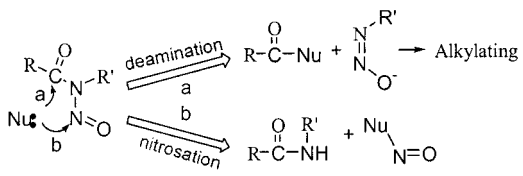
Some aliphatic *N*-nitrosamines of 7-azabicyclo[2,2,1]heptanes, such as compound **5**, undergo facile N−NO bond cleavage and are superior to the aromatic *N*-nitrosamines. They have low rotational barriers about the N−NO bond (around 15 kcal/mol).[247] Since *N*-nitrosamine is structurally analogous to an amide (Scheme 14), the N−NO bond energy is related to the partition of *p* electrons on nitrogen to the N═O π-system. A smaller rotation barrier corresponds to a lower N−NO bond energy.

Scheme 14



Type II nitrosamines have two reaction pathways. One pathway involves nucleophilic attack at the carbon of C═O to generate a tetrahedral intermediate which decomposes to an active diazotate ion (R−N═N−O^{-}). Another pathway involves nucleophilic attack on the nitrogen of the nitroso group resulting in denitrosation (Scheme 15). The nucleophile can be a biologically prevalent thiol; therefore, type II compounds are often used as NO donors for the formation of *S*-nitrosothiols.[248,249]

Scheme 15



In general, type II compounds show greater NO-releasing ability than type I *N*-nitrosamines. This can be explained by the electronic repulsion between carbonyl oxygen and nitroso oxygen or the attraction of the lone-pair electrons at nitrogen by carbonyl group, both weakening the N−NO bond.

3. Applications

N-Nitrosamines have been shown to be inhibitors of cysteine-containing enzymes. For example, dephostatin and other *N*-methyl-*N*-nitrosoanilines (**1**) were found to be the inhibitors of protein tyrosine phosphatases, papain, and caspase.[250−252] Inhibition results from the *S*-nitrosation of the critical cysteine residues in the active sites of the enzymes by the nitrosamines. Compounds **6** and **7** have been found to inhibit thrombus formation in arterioles and venules of rats,[244] while *N*-nitrosamide **9** exhibited vasodilation and mutagenicity as a result of NO release.[253] *N*-Aromatic *N*-nitrosourea **10**, which decomposes at ambient temperature without photoirradiation, showed similar NO-generating ability to that of SNAP.[254] The *N*-methyl-*N*-nitrosourea Streptozocin (STZ, **11**) is an antibiotic with diabetogenic, carcinogenic, and antitumor activity. It was concluded that NO released by STZ induced the pancreatic beta cell death.[255] *N*-Methyl-*N*-nitrosotoluene-*p*-sulfonamide (MNTS, **12**) was found to be a potent antimicrobial agent against fungi as well as Gram-positive and Gram-negative bacterial strains.[256] MNTS has also been used as a nitrosating agent.[257] A number of *N*-nitroso carbamate insecticides, including **13**, are very potent bacterial mutagens and cell-transforming agents.[258] Nitrosoguanidine MNNG (1-methyl-3-nitro-1-nitrosoguanidine, **14**) and related compounds have been shown to be vasodilators in vitro and blood pressure lowering agents in vivo.[231] Some *N*-nitrosoureas, which serve as direct alkylating agents, such as 2-chloro-ethyl-nitrosoureas, have been used as anticancer agents.[259,260]

The major deficiency of using Type II *N*-nitroso compounds as NO donors is their instability in aqueous solution and the possible side reactions that generate potent alkylating agents (the deamination pathway). Besides DNA alkylation, *N*-nitroso compounds can exert genotoxicity through transfer of the nitroso group to nucleophilic sites on the purine bases.[261] *N*-Nitrosoindole (**8**) can cause depurination, deamination, and the formation of a novel guanine analogue, oxanine.[262] These pathways have been suggested to explain the NO-like biological effects induced by nitrosated tryptophan residues in serum albumin and model dipeptides.[263]

E. *N*-Hydroxy-*N*-nitrosamines

N-Hydroxy-*N*-nitrosamines, also known as the *N*-oxy-*N*-nitrosamines, can decompose under physiological conditions to release NO. Three important *N*-hydroxy-*N*-nitrosamines are Cupferron, alanosine, and dopastin (Figure 8). They are potent antihypertensive agents[264] and inhibitors of platelet aggregation.[265] Keefer's group disclosed a series of structurally innovative *N*-oxy-*N*-nitrosamines such as **15**.[266] These compounds are heat-stable in solution

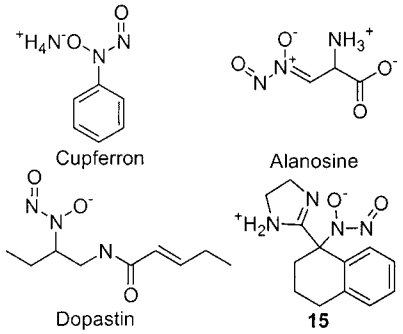


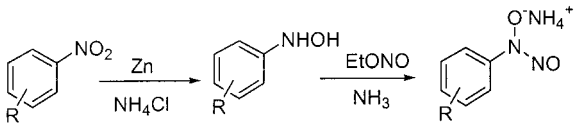
Figure 8.

and are slow NO releasers. More importantly, the decomposition to carcinogenic nitrosamines is excluded.

1. Synthesis

N-Hydroxy-*N*-nitrosamines are usually prepared by the nitrosation of the corresponding *N*-hydroxyamines.[267−269] Hydroxyamines are readily obtained by the reduction of the corresponding nitro compounds. Scheme 16 illustrates the synthesis of Cupferron

Scheme 16



derivatives. Alternative methods include the following: (1) the reaction of Grignard reagents with NO followed by treatment with ammonia or other bases,[270] (2) oxygen-free NO reacts with quinone dioximes to form salts of di-*N*-nitrosoaryl-dihydroxylamines,[271] and (3) nitrobenzene reacts with hydroxylamine and to get Cupferron directly.[272]

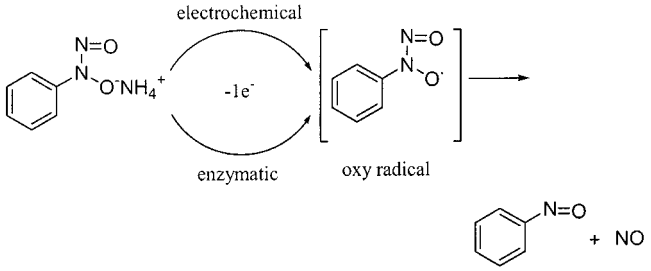
2. Properties

Cupferron has been extensively used in the field of separation, precipitation, and colorimetric analyses of metals.[273] Crystalline ammonium salt of Cupferron is freely soluble in water. At room temperature, Cupferron is relatively stable both in the solid state and in solution, generating very little NO. However, Cupferron can be thermally or photochemically decomposed to an azoxy compound and NO.[274] It is also found that Cupferron could release NO under enzymatic,[275] electrochemical,[276] as well as chemical oxidation.[277,278] During the one-electron oxidation step, Cupferron is oxidized to the unstable oxy radical, which spontaneously decomposes to nitrosobenzene and NO (Scheme 17).[270]

3. Recent Development of N-Hydroxy-N-nitrosamine Type NO Donors

Over the past few years there has been a growing interest in the development of Cupferron derivatives with the aim to search for novel NO donors. Cupferron and its derivatives have an NONO moiety attached directly to carbon instead of an oxygen or a nitrogen atom. The advantage of this type of NO donor is that after NO release, the byproducts can

Scheme 17

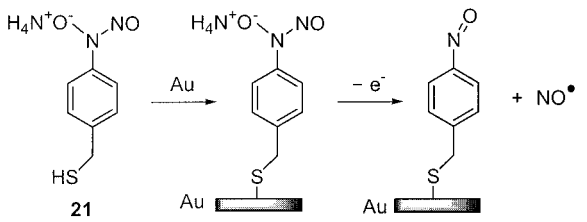


be selected to be noncarcinogenic.[268,276,279] Fine-tuning of the NO donor structure allows for preparation of novel donors with specific targeting effects. *Para*-substituted cupferrons (**16**) constitute a set of redox-sensitive NO donors.[269] These compounds release NO via spontaneous dissociation during one-electron oxidation. Electron-withdrawing groups can increase the oxidation potential and make NO release easier. *Ortho*-substituted derivatives of Cupferron (**17**) are good donors both in vitro and in vivo. These compounds show faster decomposition rates than cupeferron because the *ortho*-substitution prevents the NONO moiety from becoming planar.[268] The instability of cupeferron and its *ortho*- and *para*-substituted derivatives can be a liability in the pharmaceutical realm where targeted delivery is crucial to the success of NO donor drug efforts. In general, the preferred substituent groups attached to $-N(O^-)NO$ are α-naphthyl and *ortho*-substituted phenyl or heterocyclic aromatic rings. These groups can also be biologically active moieties, such as progesterone, estrogen, epinephrine, or other catecholamines, which can be designed to target the NO-releasing agent to a specific organ or tissue.[280]

O-Alkylated derivatives of Cupferron (**18**, **19**) and neocupferron (**20**) have also been synthesized. These analogues are more stable than their parent compounds and can be used to deliver NO photochemically, enzymatically, and chemically in controlled manners.[281,282]

Recently, Hou et al. developed a method that controlled the generation of nanomolar amount of NO.[283] The approach utilized a self-assembled monolayer of *N*-nitroso-*N*-oxy-*p*-thiomethylbenzeamine ammonium salt (**21**) bound to a gold electrode via a thiol linkage. When an electric potential was applied, one-electron electrochemical oxidation led to the release of NO (Scheme 18). There was a linear relationship

Scheme 18



between the amount of NO generated and the area of the electrode, indicating that the amount of NO release could be controlled by selecting an appropriately sized electrode surface area. This approach may

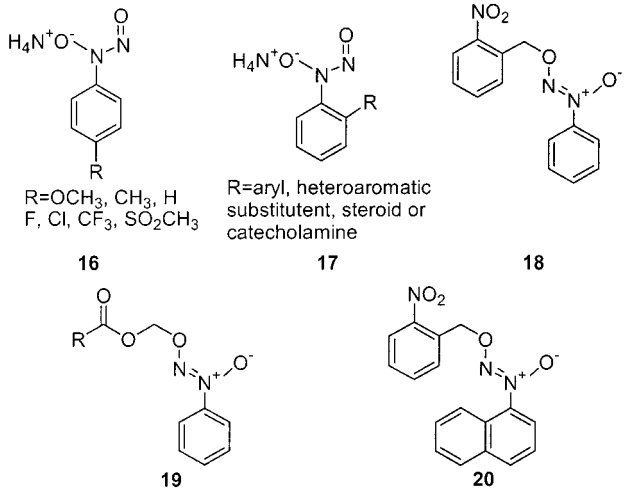


Figure 9.

be used in microelectrode arrays for biochemical applications.

F. *N*-Nitrosimines

Most nitrosimine-type NO donors are heterocyclic compounds. They are the *N*-nitrosation products of their corresponding imines. Several types of nitrosimines have been reported such as 1,3-disubstituted nitrosiminobenzimidazoles (**22**),[284] 1,3,4-thiadiazole-2-nitrosimines (**23**),[285] benzothiazole-2(3H)-nitrosimines (**24**),[286] thiazole-2-nitrosimines (**25**),[287] oligonitroso sydnonimines (**26**),[288] 3-alkyl-*N*-nitrososydnonimines (**27**),[289−291] and 2*H*-1,3,4-thiadiazine nitrosimines (**28**) (Figure 10).[292] These nitrosimines

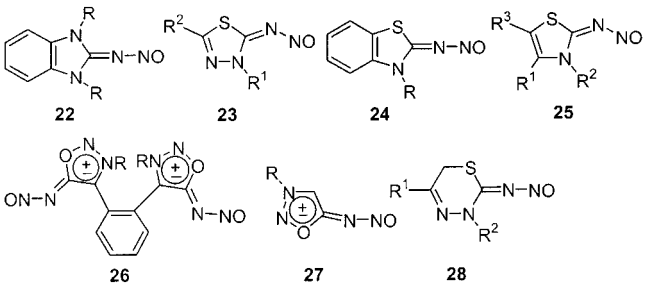


Figure 10.

have the ability to inhibit platelet aggregation in vitro. Some of them also exhibit antithrombotic and blood pressure lowering abilities in vivo.[290,293]

Nitrosimines are relatively stable when dissolved in aqueous buffers, kept cool, and protected from light. They release both NO and N_2O, the latter is via a nitroxyl intermediate (HNO). In the presence of thiols, formation of N_2O increases at the expense of NO production, which suggests that NO release involves an oxidative mechanism. However, nitrososydnonimines can release up to 2 mol of NO per molecule, one from the nitrosimine and the other from the resulting sydnonimine.

Nitrososydnonimines and thiazole-2-nitrosimines are also susceptible to photolytic cleavage of the C=N−NO bond. In aqueous solution the corresponding sydnonimine salts are formed in 90% yield at 37 °C. Only at higher temp (70 °C) is ring opening observed. In methanol solution about 25% of sydnones are

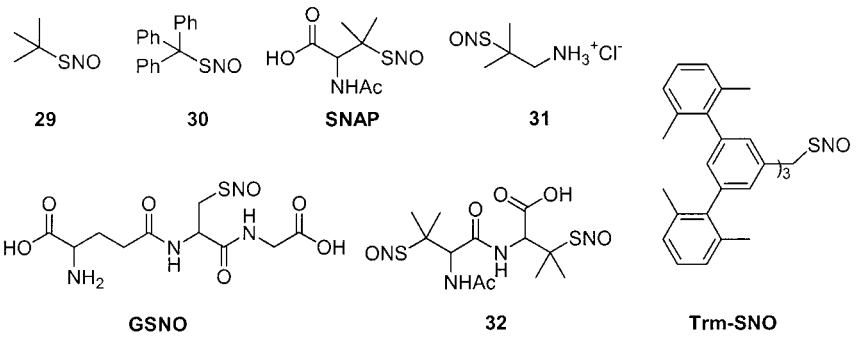


Figure 11.

obtained. The formation of N_2O from nitrososydnonimine is increased up to 11-fold by the addition of glutathione while the amount of NO is decreased. In the presence of light and thiols, soluble guanylate cyclase (sGC) is activated. These observations suggest that the nitroxylate anion NO^- might play an important role in the stimulation of sGC.[293]

G. Nitrosothiols (RSNOs)

Nitrosothiols (RSNOs) were first synthesized in 1909.[294] Most RSNOs are unstable compounds, especially for primary and secondary RSNOs. Some relatively stable RSNOs are shown in Figure 11. RSNOs have been proposed to be potential NO storage, transfer, and delivery vehicles. Many NO-related biological functions have been directly associated with RSNOs. In the past few years, several good reviews on RSNOs have been published.[295−302]

1. Synthesis

The syntheses of RSNOs involve the reaction between thiol (RSH) and NO derivatives such as NO_2 (N_2O_4), N_2O_3, and NO_2^-. NO itself reacts with thiols yielding disulfide rather than RSNOs.[303] If NO is used in the presence of oxygen or other oxidants, the *S*-nitrosation may be induced by oxidation of NO to NO_2, N_2O_3, NO_2^-, or NO^+.[304,305]

Nitrosation by NO_2^- in acid media may be the most widely used synthetic application among all the methods.[306−312] In this reaction, a strong steric effect is noted: bulky proteins and tertiary thiols react much slower than simple thiols containing easily accessible SH groups.[299] Furthermore, this reaction can be accelerated by the presence of carboxylic groups in the substrates, via an intermediate nitrosyl carboxylate.[313,314]

RSNOs can also be prepared from the reaction of their parent thiols with nitrogen oxides (NO_2, N_2O_4, N_2O_3).[315−317] Recently, this method was modified by using a new reagent [NO(18-crown-6)]NO_3/HNO_3.[318] This compound can react with RSH to give RSNOs in good yield.

The third method used to synthesize RSNOs is the reaction of thiols with alkyl nitrites. *tert*-Butyl nitrite and ethyl nitrite are the most often used agents in this application. They are mild enough to carry out selective *S*-nitrosation in the presence of primary amino groups.[319,320] Furthermore, this reaction is pH-dependent. The reaction rate increases steeply with an increase in pH until pH 10, where the rate constant levels off. It suggests that the reaction occurs via the thiolate anion.[321−322]

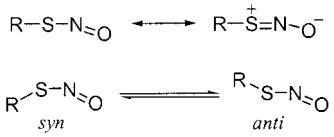
2. Physical Properties

RSNOs have characteristic UV−vis and NMR spectra. In general, they are green (for tertiary RSNOs) or red (for primary and secondary RSNOs) in color. The UV−vis spectra show three bands: two intense bands in the UV and one weak band in the visible region. The first band is in the 225−261 nm region ($\epsilon \sim 10^4$ M^{-1} cm^{-1}) which is attributed to the $\pi \rightarrow \pi^*$ transition.[323] The second band is in the 330−350 nm region ($\epsilon \sim 10^3$ M^{-1} cm^{-1}), which is attributed to the allowed $n_0 \rightarrow \pi^*$ transition.[324] The third band is in the 550−600 nm region ($\epsilon \sim 20$ $M^{-1}cm^{-1}$). It is attributed to the forbidden $n_N \rightarrow \pi^*$ transition. This band determines the compounds' color. The second and third bands of RSNOs are often used to monitor the reactions of RSNOs. In ^1H NMR and ^{13}C NMR spectra there is a downfield shift of both α-proton and α-carbon resonances upon the nitrosation of thiols. ^{15}N NMR studies of RSNOs reveal that ^{15}N chemical shifts for primary and tertiary RSNOs are around 730 and 790 ppm, respectively.[325] Electrochemical study of RSNOs indicates that RSNOs exhibit only a single diffusion-controlled irreversible reduction peak, corresponding to the release of NO from RSNOs.[326] The reduction potentials are far more negative than the reduction potential of NAD^+/NADH in vivo, which suggests that the mechanism for the RSNOs release of NO in vivo might not be via NAD^+/NADH reduction. For primary RSNOs, a linear relationship was found to exist between ^{15}N NMR chemical shifts and reduction potentials. The structures of three RSNOs have been crystallographically characterized so far; they are SNAP, Ph_3C-SNO, and Trm-SNO.[327]

The S−NO bond in RSNO exhibits significant double-bond character due to the delocalization of electron pairs at sulfur in the nitroso group. This double-bond character of the S−N bond allows RSNO to exist as two isomers, i.e., syn and anti (Scheme 19).[328] The preference for either conformation depends on the aliphatic substitution;, the syn conformation is preferred in the case of primary and secondary aliphatic groups, whereas tertiary alkyl substitution such as in SNAP prefers the anti conformation.

The bioactivity of RSNOs was previously attributed to the homolysis of the S−NO bond, where thiyl

Scheme 19

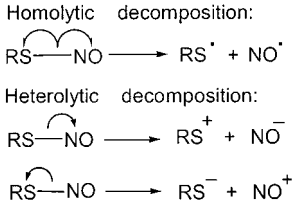


radical and NO were generated. Recently, the S−NO bond energies of RSNO have been investigated by thermodynamic and kinetic experiments as well as theoretical calculations.[329,330] It shows that the homolytic bond dissociation energies of S−NO are around 30 kcal/mol, which is prohibitively high for spontaneous thermal homolysis. These results indicate that thermal homolysis of the S−NO bond is not an important contributor to RSNO decomposition under physiological conditions.

3. Reactions

Decomposition of RSNOs. Decomposition of RSNO involves both homolytic and heterolytic cleavage of the S−NO bond. NO is released in the former decomposition (Scheme 20) and NO^+ or NO^- in the

Scheme 20

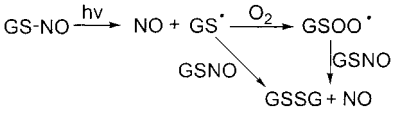


latter. It has been suggested that the formation and decomposition of low molecular weight RSNOs, such as GSNO and *S*-nitrosocysteine (CysNO), may represent a mechanism for the storage and transport of NO in vivo.[331] The breakdown of the S−NO bond can be induced by heat, UV light, certain metal ions, superoxide, and seleno compounds.

Thermal Decomposition. Most RSNO compounds are unstable at room temperature and decompose automatically to give the corresponding disulfides and NO. Thermal decomposition of RSNO is believed to be a two-step process. The first step is S−NO bond homolytic cleavage to form an NO radical and a thiyl radical (RS•), followed by dimerization of thiyl radical to form disulfide (RSSR). Compared to primary alkyl RSNOs and aryl RSNOs, tertiary RSNOs, such as SNAP, have a much higher stability. Ab initio calculations on model RSNO and RSSR indicate that one factor responsible for the high stability of SNAP is the steric interactions in the dimerization.[332] The thiyl radicals, with smaller steric hindrance, are easier to dimerize, leading to faster RSNO decomposition. Another study on the stability of RSNO compounds also suggested that the stability of RSNOs was highly substituent dependent.[333]

Photodecomposition. RSNOs are photosensitive, especially to UV light. Irradiation of GSNO at the absorption band at either 340 or 545 nm results in the release of NO and thiyl radicals.[334] In the oxygen-free environment, the thiyl radical formed will react directly with GSNO to form a disulfide GSSG and NO. However, in the presence of oxygen, GSOO• radical is first generated, followed by the reaction with another GSNO to form GSSG and NO. Therefore, NO can be produced not only via GSNO homolysis, but also from the reactions of GSNO with GS• and GSOO• (Scheme 21). Biological studies revealed

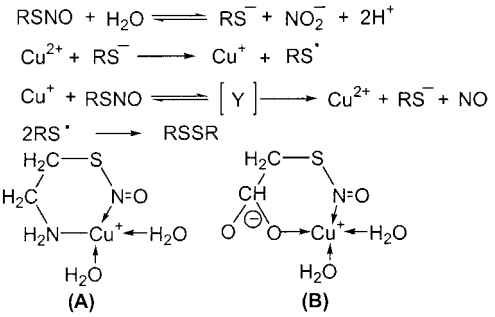
Scheme 21



that photolytic release of NO from GSNO resulted in an enhanced cytotoxic effect of GSNO on HL-60 leukemia cells.[335] Photosensitizers can promote the release of NO from RSNO.[336] Irradiation of GSNO at 550 nm in the presence of Rose Bengal resulted in a 9-fold increase in the quantum yield of NO as well as an increase in the thiyl radical formation rate.

Metal-Ion-Catalyzed Decomposition. Decomposition of RSNOs in solution can be catalyzed by metal ions such as Cu^+, Fe^{2+}, Hg^{2+}, and Ag^+.[337−340] Since the human body contains 0.1 g of copper per 75 kg of body weight and it is widely distributed in the blood, bone, and muscle, the decomposition of RSNO catalyzed by Cu has drawn the most attention. Williams et al. extensively studied the copper-ion-catalyzed RSNO decomposition and proposed a possible reaction mechanism,[337−342] shown in Scheme 22. The true

Scheme 22



catalyst is Cu^+, which is formed from the reduction of Cu^{2+} by thiolate generated by hydrolysis of RSNO or free thiol. The Cu^+ can then catalyze RSNOs decomposition through a complex intermediate Y, proposed as structure A and B based on the structure of RSNO. Both Cu^{2+} and RS^- are regenerated and are present in catalytic quantities. In the presence of metal-ion chelators, such as EDTA, RSNO decomposition is virtually halted by formation of EDTA−Cu^+ complex.

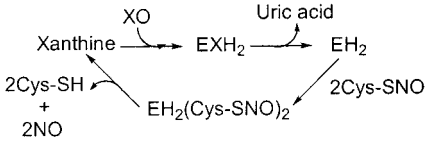
Compared to Cu^+-catalyzed RSNO decomposition, mercury(II)-catalyzed RSNO decomposition generally lacks structural dependence.[343] For example, *N*-acetylation of many cysteine derivatives can cause a significant rate reduction (10^3-fold) in the copper-ion-catalyzed decomposition. However, the same reduction does not occur for mercury(II). Experimental results suggest that in mercury(II)-catalyzed reactions the Hg^{2+} binds exclusively to the sulfur atom.

Some copper-containing enzymes such as copper−zinc superoxide dismutase (CuZn−SOD) can also catalyze the decomposition of GSNO in the presence

of glutathione (GSH).[344] It has been reported that cells such as erythrocytes and hepatocytes may contain as much as 10−30 μM CuZn−SOD[345,346] and extracellular CuZn−SOD may contribute up to 70% of the total SOD activity in both pulmonary and systemic arteries.[347] CuZn−SOD-catalyzed GSNO decomposition resulted in a sustained production of NO. Again, the true catalyst is Cu^+, generated via the reduction of the enzyme-associated Cu^{2+} in CuZn−SOD by GSH. Other superoxide dismutases such as manganese superoxide dismutase (Mn−SOD) do not exhibit the same ability to decompose GSNO. CuZn−SOD-catalyzed RSNO decomposition may represent an important physiological decomposition mechanism of low molecular weight RSNOs in vivo.

Superoxide-Catalyzed Decomposition. Recent studies indicated that xanthine oxidase (XO), a superoxide generator, in the presence of purine substrates and molecular oxygen, could induce CysNO and GSNO decomposition under aerobic conditions by $O_2^{-\bullet}$ dependent and independent pathways.[348,349] GSNO decomposition is fully dependent on a second-order reaction with $O_2^{-\bullet}$. Superoxide-catalyzed CysSNO decomposition can also undergo another enzymatic pathway in which XO first initiates the oxidation of xanthine (XH_2) to uric acid (UA), resulting in a reduced enzyme (EH_2) (Scheme 23). Once

Scheme 23



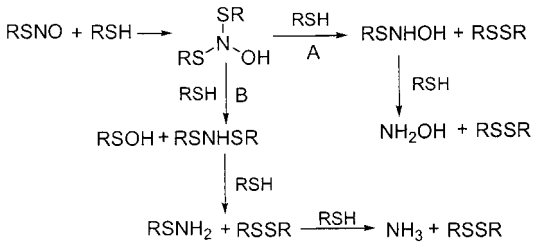
CysNO binds to EH_2 to form a complex $EH_2(CysSNO)_2$, NO is released following an electron transfer to CysNO from the flavin site. The measured rate constant of the reaction of RSNO with superoxide is several orders of magnitude smaller than the diffusion-controlled reaction of superoxide with NO (to give peroxynitrite), and RSNOs may thus provide a storage place in biological systems for NO, protecting it from rapid decomposition by superoxide. These findings suggest the possibility of the involvement of superoxide in the metabolism of RSNOs.

Seleno Compounds and Glutathione Peroxidase-Catalyzed Decomposition. It is well documented that selenium is an essential mineral in the mammalian diet. Selenium deficiency is tightly associated with many heart diseases such as myocardial necrosis and atherosclerosis.[350] Recent studies by Freedman indicate that glutathione peroxidase (GPx), an essential selenium-containing antioxidant enzyme, is involved in the inhibition of platelet aggregation by RSNOs.[351] The study suggests that GPx catalyzes the metabolism of endogenous GSNO to liberate NO in the presence of H_2O_2. Wang and co-workers found that diselenides could also catalyze the decomposition of RSNO to produce NO.[352] The initial activation is believed to involve the interchange reaction of diselenide and thiol. The selenol generated from this exchange goes on to react with RSNO to release NO.

Reactions with Thiols. Early experiments showed that an exchange of the NO group occurred when RSNO was mixed with RSH.[353−357] If a relatively unreactive (toward NO formation) RSNO is treated with a thiol, the "new" RSNO formed by transnitrosation might, for structural reasons, be much more reactive toward NO formation. This may provide a more rapid alternative method for NO formation. The kinetics and mechanistics of NO transfer between RSNO and thiols at different conditions have been extensively explored by Williams and co-workers.[358−361] It was found that at increased pH values, NO transfer was faster. At low thiol concentrations, the addition of Cu^{2+} resulted in a large rate increase. Consequently, the addition of EDTA or the specific Cu^+ chelator neocuproine completely suppressed the reaction. However, at high thiol concentrations, the reaction rate was unaffected neither by the removal of adventitious metal ions nor by the addition of Cu^{2+}. Kinetic studies on NO transfer from seven different RSNOs to Cys34 of BSA showed that the rate constant obtained for the reaction of BSA with *S*-nitrosomercaptoethylamine was about 10 times faster than that for the reaction of BSA with CysNO and 40 times faster than that of the reaction between BSA and GSNO.[362] These results indicate that the transnitrosation reaction is not only affected by the structure of RSNO, but also by the overall anionic charge in the reaction system.

Besides transnitrosation, a variety of other intermediates and products are observed in the reaction of RSNOs with thiols, depending on the reagent ratios and experimental conditions.[363−365] Those products include N_2O, NH_2OH, NH_3, disulfides, sulfinic acids, and sulfinamides. The mechanism for the generation of NH_2OH is proposed in Scheme 24, path

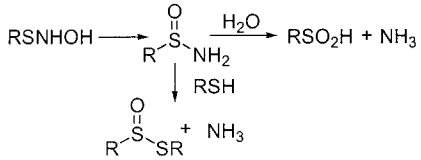
Scheme 24



A. The production of NH_3 was explained assuming a different reactivity of the addition product, RSN(OH)SR. (Scheme 24, path B)

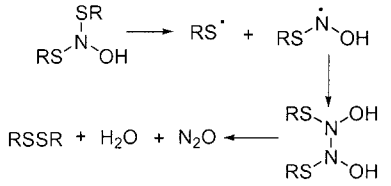
Wong et al. suggested that *N*-hydroxysulfinamide undergoes an intramolecular rearrangement followed by hydrolysis or reaction with RSH to produce NH_3[363] (Scheme 25). This proposed mechanism is supported by GCMS results.

Scheme 25



The formation of N_2O is explained in terms of the homolytic splitting of *N*-hydroxysulfinimide, followed

Scheme 26



by radical recombination and decomposition of the dimer (Scheme 26).

Since the reaction of RSNOs with thiols often leads to the formation of a disulfide bond, site-specific *S*-thiolation is emerging as a novel mechanism by which RSNOs may modify functionally important protein thiols. Recently, Klatt et al.[366] developed a GSNO−Sepharose conjugate. This new reagent mimics site-specific *S*-glutathionylation of cysteine-containing proteins by free GSNO in vitro. With few exceptions, protein binding to this matrix correlates well with the susceptibility of the investigated proteins to undergo GSNO-induced mixed-disulfide formation. This is a potentially useful tool in isolating and identifying target protein candidates for RSNO-induced mixed-disulfide formation.

Reaction with Ascorbate. Ascorbate can reduce Cu^{2+}, making it effective for the decomposition of RSNOs. However, there is evidence that shows ascorbate promotes NO production from GSNO in blood plasma, even in the presence of metal ion chelators.[367,368] On the basis of these observations, Williams et al. examined this system in detail.[369] They found that ascorbate reacted with RSNOs in two separate reactions;[370] both of them led to NO formation: (I) when ascorbate is a reducing agent for Cu^{2+}, which happens at low ascorbate concentration ($\sim 10^{-4}$ M), disulfide will be the major product; (II) when ascorbate acts as a nucleophile, it will directly undergo electrophilic nitrosation, leading to NO and thiol formation. This reaction is dominant at higher ascorbate concentration ($\sim 10^{-3}-10^{-2}$ M). The thiol product was found to be essentially quantitative only from the reaction with the higher ascorbate concentration. On the basis of these results, ascorbate has been used as an efficient probe in distinguishing the formation of *S*-NO and -*S*−*S*- bonds in some cysteine-dependent enzymes.[371,372] If modification of the free thiol group in the enzyme formed *S*-NO, the addition of ascorbate could reduce the *S*-NO moiety to free thiol, hence reactivating the enzyme. If modification of the free thiol group in the enzyme formed a mixed disulfide (-*S*−*S*-), then the addition of ascorbate would not reduce it to free thiol, leaving the enzyme inactive.

4. Development of Novel RSNOs

RSNOs represent a unique type of NO-releasing molecules. Current interest in RSNO has focused on the designs and syntheses of novel RSNOs with optimized pharmacokinetic properties.

Sugar-SNAPs. A series of sugar-*S*-nitrosothiols (sugar-SNAPs), developed as novel NO-donating agents by Wang and co-workers, have shown promising pharmacokinetic properties.[373−376] Some typical compounds are shown in Figure 12. These compounds

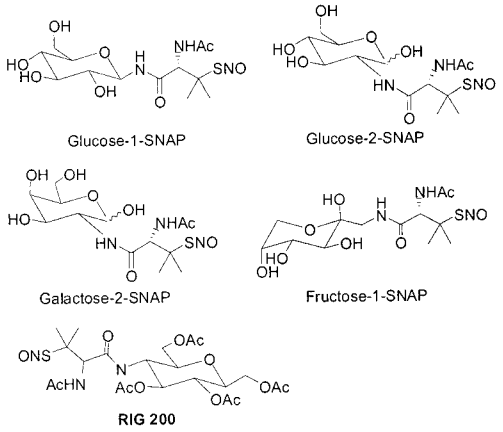


Figure 12.

were designed based on the observations that facilitated transport of monosaccharides in mammalian cells was accomplished by the glucose transporter family of transmembrane properties.[377,378] They are constructed by an aglycone unit conjugated with a mono- or oligosaccharide. The aglycone moiety provides the pharmacological activity, whereas the carbohydrate unit enhances water solubility, cell penetration, and drug−receptor interaction as well as influencing the dose−response relationship. Compared to SNAP, sugar-SNAPs have higher stability and slower NO-releasing properties in aqueous solutions. Preliminary cytotoxic studies of glucose-SNAPs against different cancer cells indicated that glucose-SNAPs were more potent than SNAP itself. Another sugar-SNAP derivative, *N*-(*S*-nitroso-acetylpenicillamine)-2-amino-2-deoxy-1,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranose (RIG200), was synthesized by Butler et al.[379,380] RIG200 has been found to prolong vasodilation in the endothelium-deduced, isolated rat femoral arteries.

***S*-Nitroso-1-thiolsugars.** Butler et al. also developed a series of NO donor compounds derived from 1-thiosugars, such as glucose, galactose, xylose, maltose, and lactose based on the similarity of glycerol and sugar (Figure 13). These novel NO donor com-

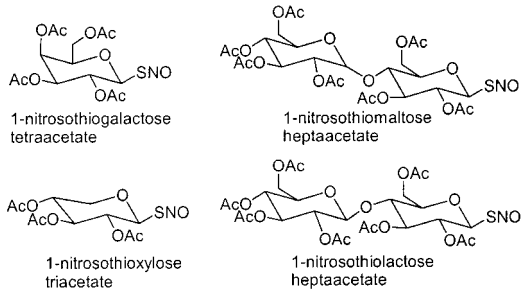


Figure 13.

pounds have both hydrophobic and hydrophilic groups which allow them to be delivered transdermally.[381] For example, *S*-nitroso-1-thio-2,3,4,6-tetra-*O*-acetyl-glucopyranose was shown to be more effective in human cutaneous vascular smooth muscle relaxation than SNAP when delivered transdermally. It suggests that this type of RSNOs can act as a better NO donor drug for human smooth muscle relaxation. In addition, it was found that the effect of copper ions

on the release of NO from *S*-nitroso acetylglucose was less than that on SNAP.

Williams and co-workers also prepared and characterized a series of novel RSNO compounds derived from thiosugar and 1-thioglycerol (Figure 14).[382]

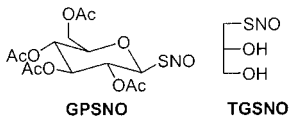


Figure 14.

GPSNO exhibited high stability, while TGSNO decomposed rapidly at room temperature.

***S*-Nitrosopeptides.** A number of peptides containing SNAP or *N*-substituted analogues of SNAP have been synthesized by Butler, Al-Sa′doni, and coworkers.[383−386] (Figure 15). The catalytic effect of

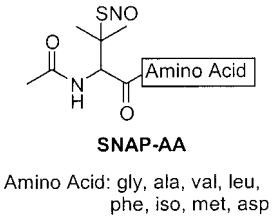


Figure 15.

copper ions upon release of NO from these compounds is considerably less than that with SNAP. On the other hand, the vasodilation by these compounds and the inhibition of hemoglobin varies quite considerably within the family. SNAP-glycine, SNAP-leucine, SNAP-proline, SNAP-aspartic acid, and SNAP-glutamic acid are more active than SNAP, SNAP-phenylalanine is less active, while SNAP-alanine, SNAP-valine, SNAP-isoleucine, and SNAP-methionine have similar activities to SNAP. These peptide RSNOs also inhibited U46619-induced platelet aggregation with similar potency to GSNO and SNAP. These findings indicate a degree of tissue selectivity that may prove to be of therapeutic usefulness.

Fluorophore-Labeled RSNOs. Mutus and Wang's groups developed a new type of RSNOs (**33**−**40**) which contained different fluorophores (Figure 16).[387,388] The fluorescence emission spectra of these RSNOs could be enhanced upon removal of the NO group either by photolysis or by transnitrosation with free thiols such as GSH. The fluorescence enhancement was reversible in that it could be quenched in the presence of excess GSNO. Attempts have been made to utilize such compounds as an intracellular probe of thiols/*S*-nitrosothiols. Fluorescence microscopy of fibroblasts in culture indicated that the intracellular *N*-dansyl-*S*-nitrosohomocystein (**33**) reached a maximum within 5 min. The fluorescence was directly proportional to intracellular GSH levels. The fluorophore-labeled RSNO-preloaded cells were sensitive to GSNO uptake since the intracellular fluorescence decreased as a function of time upon exposure to extracellular GSNO. These compounds could be used to study the kinetics of SNO/SH

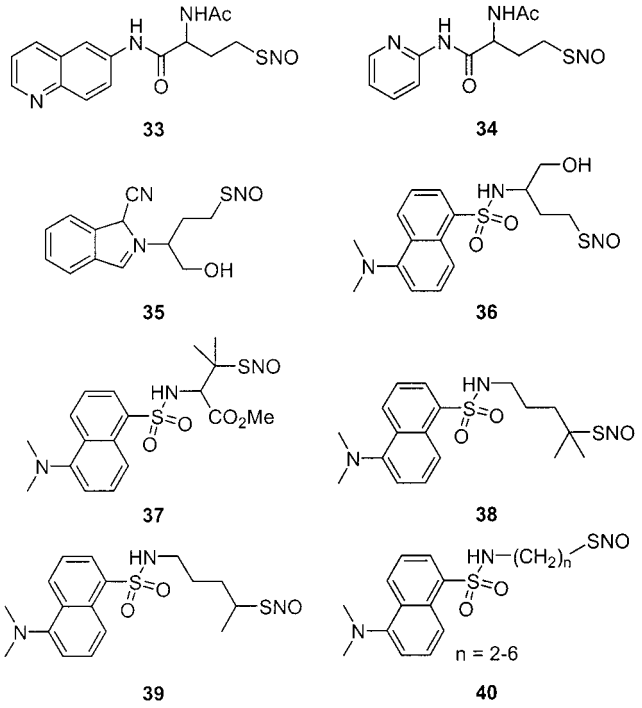


Figure 16.

transnitrosation as well as probe for the inhibition mechanism of RSNOs on cysteine-containing enzymes.[389]

5. Biological Applications

Although the biological activities of RSNOs were known even before the physiological functions of NO were realized,[390−392] only after the role of NO in biology was established did these molecules attract much more attention than before. So far, many reports have revealed the unique relationship between RSNOs and NO. For example, it was shown that RSNO was the intermediate of the function of some nitrovasodilators such as nitroglycerin and 3-morpholinosydnonime.[393] RSNOs themselves can be used as potent antiplatelet agents and vasodilators. These functions are usually attributed to NO release, although some results also suggested that RSNOs can exhibit direct effects without NO generation.[394,395] If the catalysts for RSNO decomposition exist in the system, such as copper ion and ascorbate, RSNOs generally behave as NO donors in their activities.[396−399]

Other biological functions of RSNOs include their protection against cellular toxicity associated with oxidative stress.[400] The mechanism involves the generation of NO, followed by a radical−radical termination of NO with a propagating free radical.[401−403] SNAP and GSNO were also shown to protect the lung epithelium from oxidant-induced increases in monolayer permeability as well as the protection of endothelial cells from the toxic effects of oxidized low-density lipoprotein.[404,405] RSNOs can also inactivate aconitase and inhibit the uptake of norepinephrine in sympathetic neurons.[406,407]

Besides GSNO and SNAP, another important RSNO is *S*-nitrosohemoglobin. This molecule represents an oxygen-sensitive modulator of vascular tone.[408,409] It acts as a vasoconstrictor in high con-

centrations of oxygen; while in low oxygen conditions, its vasoconstrictive effects are substantially diminished.[410] Glutathione can convert *S*-nitrosohemoglobin from a vasoconstrictor to a vasorelaxor. However, glutathione has no effect on vascular constriction by oxyhemoglobin. Recently, the kinetics of transnitrosation between GSNO and hemoglobin have been studied.[411,412] It was shown that this transnitrosation is slow (~0.1 M^{-1} s^{-1}). The equilibrium constant for transnitrosation from GSNO to oxyhemoglobin was close to unity, which suggested that the nitroso group is evenly distributed among glutathione and hemoglobin in the fully oxygenated state. A major effect of *S*-nitrosation of hemoglobin is to increase its oxygen affinity, making the *S*-nitrosated subpopulation of hemoglobin preferentially oxygenated at low oxygen tension.

H. *C*-Nitroso Compounds

C-Nitroso compounds contain a nitroso group attached to a carbon atom. To date, only tertiary *C*-nitroso compounds, such as 2-methyl-2-nitrosopropane (MNP) and compounds that are geminally substituted with electron-withdrawing groups, have been examined as NO donors (Figure 17).

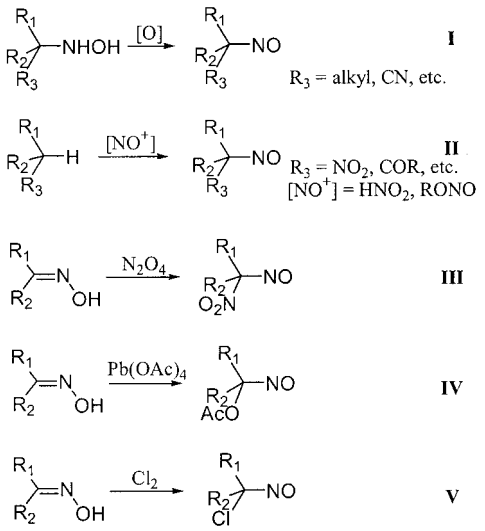
41a: R_1, R_2, R_3 = Me (MNP)
41b: R_1, R_2 = alkyl, R_3 = NO_2
41c: R_1, R_2 = alkyl; R_3 = COR
41d: R_1 = alkyl; R_2, R_3 = COR
41e: R_1, R_2 = alkyl; R_3 = CN
41f: R_1, R_2 = alkyl; R_3 = OCOR
41g: R_1 = H, R_2 = alkyl
41h: R_1 = H, R_2 = aryl
41i: R_1 = Me, R_2 = CO_2R

Figure 17.

1. Synthesis

Aliphatic *C*-nitroso compounds such as MNP and gem-cyano-nitroso compounds can be readily prepared from the corresponding hydroxylamines (Scheme 27, I).[413] Other *C*-nitroso compounds that

Scheme 27



are geminally substituted by strong electron-withdrawing groups (nitro or carbonyl group) can be obtained by either nitrosation (Scheme 27, II),[414,415] or through oxidation/addition (Scheme 27, III−V) upon oximes that are geminally substituted by nitro (pseudonitroles),[416] chloro,[417−419] and acyloxy[420−422] groups.

2. Physical Properties

Aliphatic *C*-nitroso compounds can exist as monomers, dimers (azodioxides), or tautomeric oximes. In general, the dimers and oximes are the more stable forms; however, in some cases the monomers are preferred (e.g., tertiary and gem-electron-withdrawing substitution).[423,424] The stable monomers are characterized by their blue or green color (λ_{max} 630−790 nm), due to an n → π^* transition of the NO group, as well as by an infrared stretch ($\nu_{N=O}$ 1540−1620 cm^{-1}). The activation barrier for dissociation of the dimers is ca. 20−30 kcal/mol, whereas dimerization is 6−10 kcal/mol. Aliphatic *C*-nitroso compounds are unstable. The BDE of the C−N bond in aliphatic *C*-nitroso compounds is about 36−40 kcal/mol,[425] quite close to those of the O−NO bond in nitrite esters and $O-NO_2$ bond in nitrate esters. They undergo homolytic C−N bond scission, giving rise to NO free radical and the corresponding carbon-centered radical, which subsequently combines with unreacted *C*-nitroso species to form nitroxide radical $R_2N-O^{\bullet}$.[426−433] This allows their use as potential NO donor compounds.

3. Biological Applications

Since *C*-nitroso compounds may release NO under photo/thermolysis, their potential as an NO donor has recently been explored by several research groups. Pou et al. showed that MNP (**41a**) could be used for delivering NO in a photochemically controlled manner for biochemical research. Both NO and *tert*-butyl radical were detected.[434] Illumination of MNP caused a dose- and time-dependent increase in cGMP which induced relaxation of preconstricted rat pulmonary arterial rings.

Rehse and Herpel found that some pseudonitroles (**41b**) inhibited the aggregation of blood platelets.[435] When these pseudonitroles were administered orally to rats, the thrombus formation in mesenteric arterioles and venules was inhibited up to 25%. They suggested that the generation of NO in vivo was an enzyme-supported process rather than a thermal formation of NO.

Rehse and Herpel also observed considerable in vitro antiplatelet activity in compounds **41c**, **41d**, and **41e**.[436] The dimers of **41e** were the most active. Compared to the single gem-carbonyl substitution (**41c**), an additional acyloxy or carbonyl substitution (**41d**) significantly enhanced the antiplatelet activity, suggesting the importance of strong electron-withdrawing groups in the geminal position. When administered orally to rats, all compounds inhibited the thrombus formation in mesenteric arterioles and venules. The above pharmacological effects were attributed to an NO-dependent mechanism. Di Stilo et al. observed that compounds **41b** and **41f** displayed good in vitro vasodilation and were able to increase the basal level of cGMP.[437] Their potencies as va-

sodilators decreased in the presence of oxyhaemoglobin. Surprisingly, they found that pseudonitroles **41b** exhibited clear cardiovascular effects. The haemodynamic profile of the most interesting compounds **41c,d** correlated with the NO-releasing effect of these compounds.

In the case of vicinal (β-) nitro substitution (**41h**−**j**), Rehse and Herpel observed comparatively strong antiplatelet activity;[438] compound **41i** (R_2 = Ph) was the most active compound. Replacement of the vicinal nitro group in **41j** by other less electron-withdrawing groups led to diminished activity. An NO-mediated mechanism was suggested for the observed pharmacological effects.

I. Diazetine Dioxides

Diazetine dioxides (DD) are four-membered heterocycles with a general structure as shown in Figure 18. Each general structure represents three resonance structures. Several important members of this class are listed in Figure 19. The 3,3,4,4-tetramethyl-

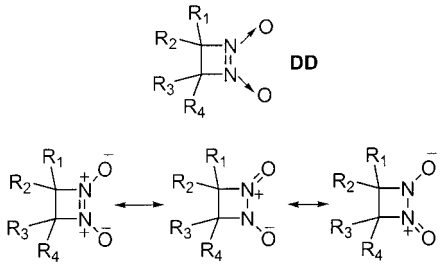


Figure 18.

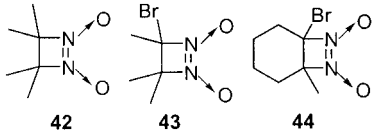


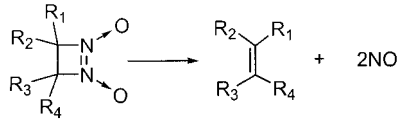
Figure 19.

1,2-diazetine 1,2-dioxide (**42**) was first reported in 1971.[439] It has a lower triplet energy than dienes and does not react through its triplet state. Compound **42** has been extensively used as a triplet quencher.[440−451] Recently, the biological activity and NO release mechanism of DD have been studied. Diazetine dioxides are prepared by the oxidation of the corresponding bishydroxyamines with NaOBr in aqueous base.[452−456] 3-Bromo-3,4,4-trimethyl-3,4-dihydrodiazete 1,2-dioxide (**43**) and 3-bromo-4-methyl-3,4-hexamethylene-3,4-dihydro-diazete 1,2-dioxide (**44**) are commercially available.

1. Reactions

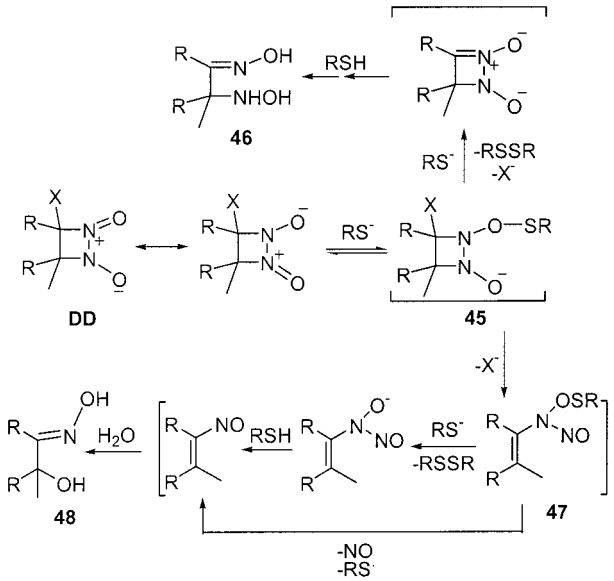
The diazetine dioxides decompose in water at physiological pH and temperature, producing two molecules of NO per DD molecule (Scheme 28).[457−461]

Scheme 28



Using nitronylnitroxides as spin traps for NO, the rate constants of DD decomposition in water and in DMSO have been determined.[462] Recently, the thiol-induced NO release from 3-halogeno-DD derivatives were reported.[463] A reaction mechanism has been proposed (Scheme 29) based on the rate of NO release

Scheme 29



that depends on the thiol concentration. Reversible nucleophilic addition of the thiolate anion at the *N*-oxide oxygen atom results in the formation of an unstable intermediate **45**. This sulfenic acid derivative reacts with thiol leading to a disulfide compound, and further reduction produces compound **46**. Another pathway is the spontaneous decomposition forming intermediate **47**, which can be reduced by thiol. Intermediate **47** undergoes spontaneously homolytic decomposition accompanied by NO formation. The resulting nitroalkene reacts with water or other nucleophiles present in the reaction mixture.

2. Applications

The diazetine dioxides were found to exert strong vasorelaxation and antiaggregation effects.[457−461] In vivo experiments performed with perfused rat tail arteries showed that some DD derivatives were highly effective vasodilators. A significant decrease of systolic arterial blood pressure was observed in hereditary hypertensive rats when DD was injected intraperitoneally.[462] The NO-dependent mechanism of guanylate cyclase activation and intraplatelet cGMP accumulation was believed to be responsible for the antiaggregatory/disaggregatory properties of these compounds. Due to the relatively high concentration of thiols within cells, the vasorelaxation is only slightly influenced by the addition of thiol to the media.[463,464]

J. Furoxans and Benzofuroxans

Furoxans (1,2,5-oxadiazole-2-oxides) and benzofuroxans represent another important class of NO donors. Several representative compounds are listed in Figure 20. There have been several comprehensive

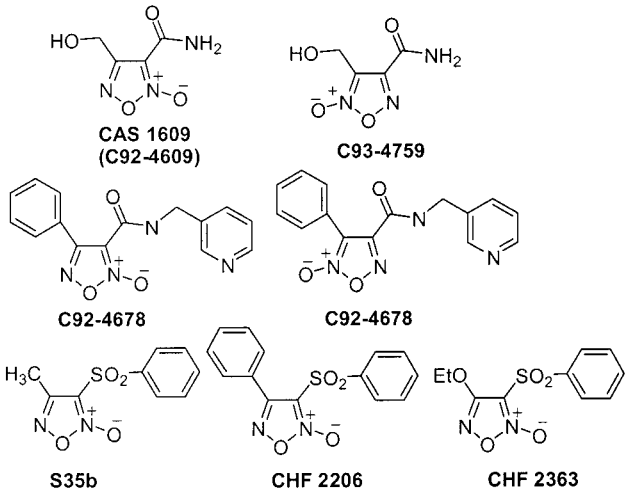


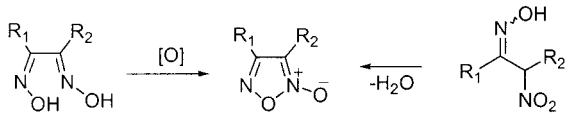
Figure 20.

reviews regarding their chemistry.[465−471] Furoxans have been shown to exert a variety of NO-related bioactivities, including cytotoxicity, mutagenicity, immunosuppression, central muscle relaxant properties, anticonvulsive effects, monoamino oxidase inhibition, and direct vasodilator and blood pressure lowering activities. Benzofuroxans can be used as in vitro inhibitors of RNA synthesis in sheep lymphocytes, being potent antileukemic and immunosuppressive drugs.[472−474] Derivatives of benzofuroxan, such as furazanobenzofuroxan, furoxanobenzofuroxan, and furoxanobenzothiadiazole, have been found to be potent in vivo and in vitro vasodilators.[475−477]

1. Synthesis

Furoxans can readily be prepared by ring closure or cycloaddition (Scheme 30). Monocyclic furoxans are

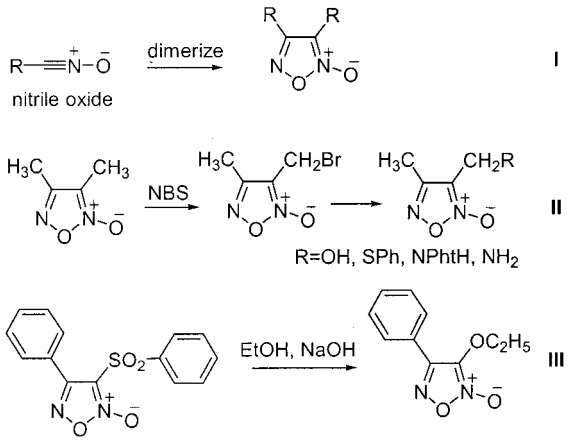
Scheme 30



most often prepared by oxidative cyclization of 1,2-dioximes. Diverse oxidizing conditions have been employed such as hypohalite,[478,479] ferricyanide, ceric ion, nitric acid and nitrogen oxides,[480] manganese dioxide,[481] lead tetraacetate, *N*-iodosuccinimide, and phenyliodine(III) bistrifluoroacetate. Another important entry to furoxans is the dehydrative cyclization of α-nitro-ketoximes with, for example, sulfuric acid, a sulfur trioxide-dimethyl formamide complex, or alumina.[482] A number of functional groups can be accommodated in addition to alkyl and aryl groups. For example, nitrosation of crotonaldehyde affords 4-formyl-3-methylfuroxan.[483]

Symmetrically substituted furoxans can be prepared by dimerization of the corresponding nitrile oxide which can be generated in situ from iminoyl chloride (Scheme 31, **I**).[484] The principal sources of nitrile oxides are oximes, hydroximoyl halides, and nitromethyl compounds. For asymmetrically substituted furoxans, careful consideration must be taken in selecting the route in order to avoid the formation of mixtures of 2- and 5-oxide isomers. One successful

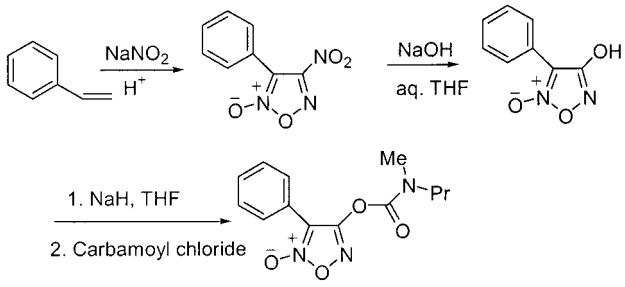
Scheme 31



approach is the nucleophilic substitution of 3-bromomethylfuroxan derivatives (Scheme 31, II)[485] or benzenesufonyl-substituted furoxans (Scheme 31, III).[486]

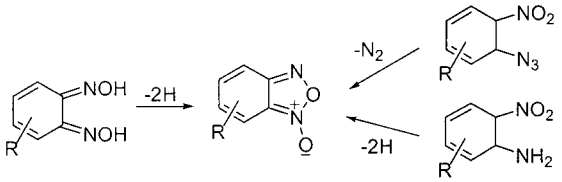
The Wieland reaction has also been used for the synthesis of furoxans.[487] A series of 4-aryl-1,2,5-oxadiazole-3-yl-*N*, *N*-dialkylcarbamate derivatives have been prepared by this method.[488] One specific example is shown in the Scheme 32.

Scheme 32



The principal methods for the synthesis of benzofuroxans involve oxidation of *O*-quinone dioxime, thermolysis of *O*-nitroaryl azides, and oxidation of *O*-nitroanilines (Scheme 33).[489−491] Benzofuroxans

Scheme 33

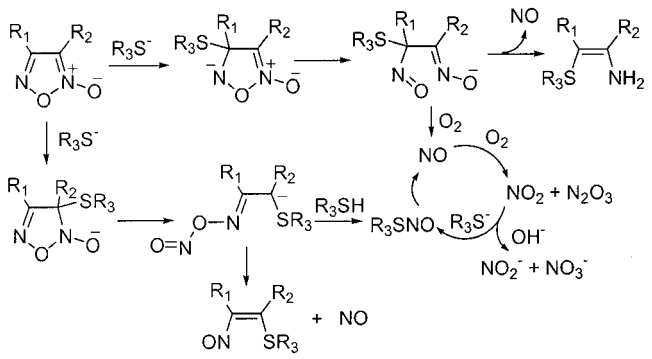


can also be formed by Boulton−Katritzky rearrangement of 7-nitro-2,1-benzisoxales and 4(7)-nitrobenzofuroxans.

2. Reactions

Furoxans are thermally stable compounds. They are also stable against acids and electrophiles. However, their stability toward bases and nucleophiles is less pronounced. The question of how they decompose to NO has attracted a lot of interest. Studies have shown that the release of NO takes place in the presence of thiols (Scheme 34).[492,493] The attack of RS^- at position 3 or 4 leads to intermediates that

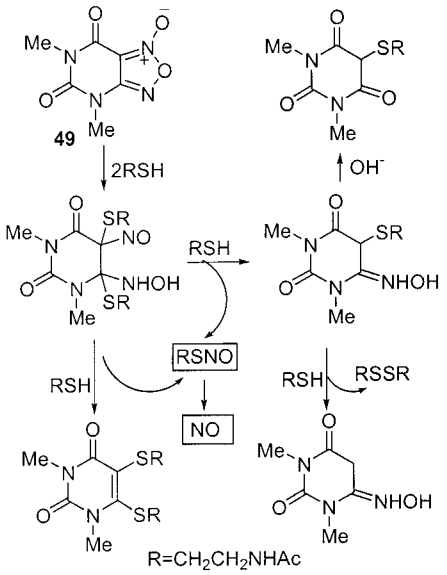
Scheme 34



undergo ring opening to the nitroso derivatives. NO is then formed by oxidation of eliminated nitrosyl anions (NO^-). The reaction of the nitroso derivative with thiol may yield a *S*-nitrosothiol that decomposes to NO via radical cleavage. The generated NO reacts with oxygen to form NO_2 and N_2O_3, which can hydrolyze to form nitrite and nitrate anions. Both anions can nitrosate thiols to form *S*-nitrosothiols and nitrite.

Another thiol-dependent release of NO from furoxans has been studied in detail using the interaction of 4,6-dimethyl-4*H*-[1,2,5]oxadiazolo[3,4-*d*]pyrimidine-5,7-dione 1-oxide (**49**) with thiols such as *N*-acetylcysteamine, cysteine, and glutathione.[494] As outlined in Scheme 35, the attack by thiol occurs at both the

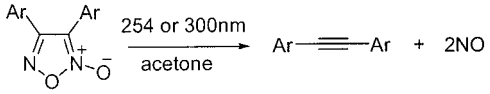
Scheme 35



3 and 8 positions, leading to subsequent ring opening. Further attack by another thiol to the C-5 nitroso group of the intermediate releases RSNO, an NO precursor, together with the formation of other products.

Recently, furoxans were found to decompose photochemically (Scheme 36). Photolysis of 3,4-bis-2′-

Scheme 36



cholorophenylfuroxan generates NO and bis-2-chlorophenylacetylene.[495]

3. Biological Applications

The R-substituted and di-R-substituted phenylfuroxans such as 3,4-dicyanofuroxan and 4-phenyl-3-furoxancarbonitrile display high vasodilatory activity on strips of rat thoracic aorta preconstricted with noradrenaline and are potent inhibitors of platelet aggregation.[491,496] The C92−4609 (CAS 1609), C92−4678, C92−4679, and C93−4759 are tolerance-resistant nitrovasodilators.[497,498] All four compounds are potent vasodilators in the femoral artery. However, they are less potent in the jugular vein by at least 1 order of magnitude. The vasodilatory potency of the furoxans correlated well with their NO-releasing capacity, which was estimated by the stimulation of purified soluble guanylyl cyclase and electron spin resonance spectroscopy with an NO trapping agent.

S35b is the most active derivative among a series of 4-methyl-3-(arylsulfonyl)furoxans for inhibition of platelet aggregation.[499] It can dose-dependently inhibit platelet aggregation evoked by arachidonic acid, collagen, ADP, and thrombin. S35b produces a significant increase in cGMP which likely causes inhibition at an early stage of platelet activation.[500] The reversal of noradrenaline-induced contraction of rabbit aortic rings by arylsulfonyl derivatives has also been reported.[501] CHF 2206 is a new potent vasodilating and antiaggregating drug.[502] It has been shown to be an inhibitor of rabbit aortic ring contraction induced by norepinephrine, the stable prostaglandin $F_{2\alpha}$ analogue, U-46619, and KCl. When preincubated with platelet-rich plasma, CHF 2206 reduced, in a dose-dependent manner, the aggregation induced by the aggregation concentration of collagen, ADP and platelet activating factor (PAF). This compound increases the platelet cGMP level that is clearly associated with the inhibition. CHF 2363 was also reported to exert a potent antiaggregation and vasorelexant activity via NO release.[503] Preincubation of CHF 2363 with human platelet-rich plasma produced a concentration-dependent inhibition of the platelet aggregation induced by collagen, ADP and platelet activating factor. The compound was a potent inhibitor of rubbed endothelium rabbit aortic ring contraction induced by noradrenaline. Increasing concentrations of CHF 2363 elevated platelet cGMP levels while not affecting the cAMP levels.

4. Development of Novel Compounds

The derivatives of 1,2,5-oxadiazole *N*-oxide and benzo[1,2-*c*]1,2,5-oxadiazole *N*-oxide (**50**−**55**) (Figure 21) have been used as potential antitrypanosomal drugs.[504] They also can be used as herbicidal agents. Many of these compounds exhibited moderate to good herbicidal preemergence activity against *triticum aestivm*. The most active compound, butylcarbamoylbenzo[1,2-*c*]1,2,5-oxadiazole *N*-oxide, displayed herbicidal activity at a concentration as low as 24 g/ha.[505]

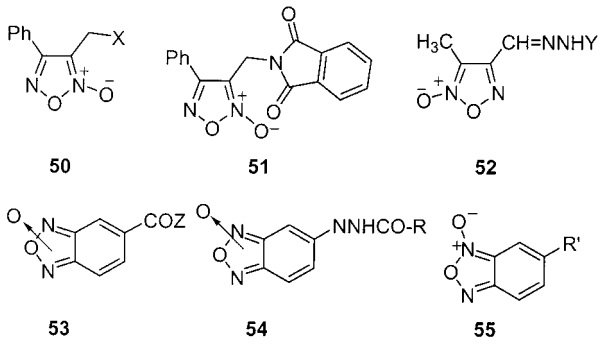


Figure 21.

In 1997, G. Sorba et al. reported a series of water-soluble furoxans (**56**−**61**) (Figure 22).[506] All of these

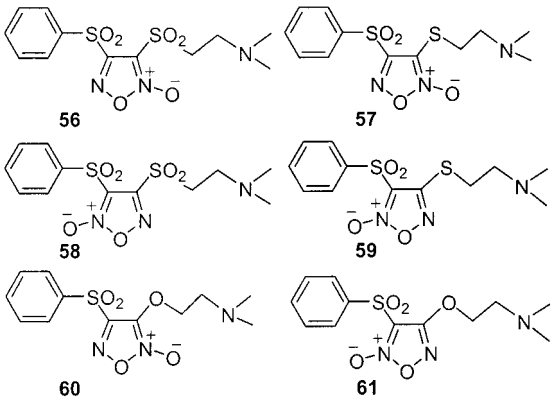


Figure 22.

compounds released NO when treated with a large excess of cysteine under physiological conditions. The compounds were able to bring about a concentration-dependent relaxation of the endothelium-denuded strips of rat aorta precontracted with noradrenaline, Figure 23.

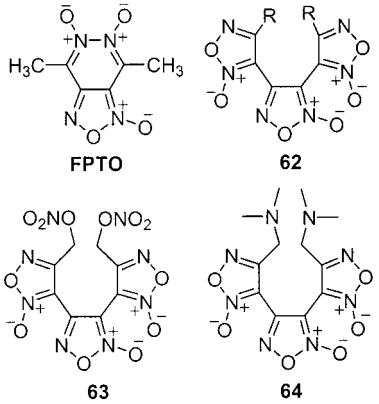


Figure 23.

The pyridazine di-*N*-oxide 4,7-dimethyl-1,2,5-oxadiazolo[3,4-*d*]pyridazine 1,5,6-trioxide (FPTO) relaxes noradrenaline-precontracted aortic rings. It is also a potent inhibitor of ADP-induced platelet aggregation. FPTO significantly increased cGMP levels in aortic rings and platelets. The vasorelaxant activity of FPTO is sGC-dependent, and a predominant role is played by NO at FPTO concentration below 1 μM. However, inhibition of platelet aggregation is only partially related to sGC activation.[507] The trimeric furoxans (**62**−**64**) display high vasodilating potency; the most active one is 5−10 times as potent as GTN. The potency decrease observed in the presence of HbO_2 agrees with the involvement of NO in the vasorelaxing action.[508]

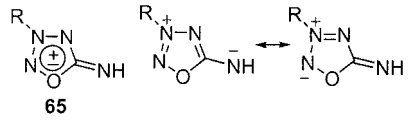


Figure 24.

K. Oxatriazole-5-imines

The mesoionic oxatriazole-5-imines (**65**) (Figure 24) are structurally isosteric to sydnonimines, comprising another important class of NO-releasing agents. The first compound, 3-cyclohexyl-1,2,3,4-oxatriazole-5-imine hydrochloride, was reported in 1965.[509] Five years later, Masuda et al. reported the application of oxatriazole-5-imines and their derivatives as a blood pressure depressant.[510,511] However, the extensive investigation of NO-releasing properties and biological application of oxatriazole-5-imines began only about 10 years ago. Figure 25 shows several

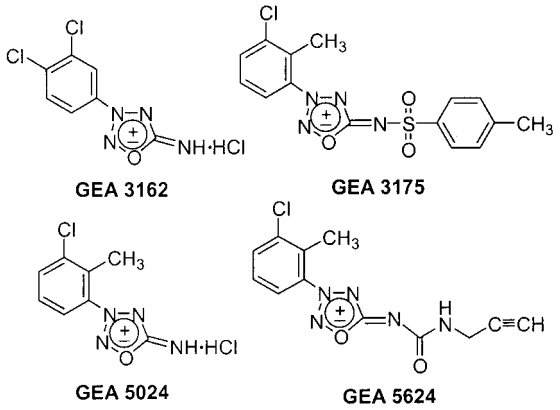


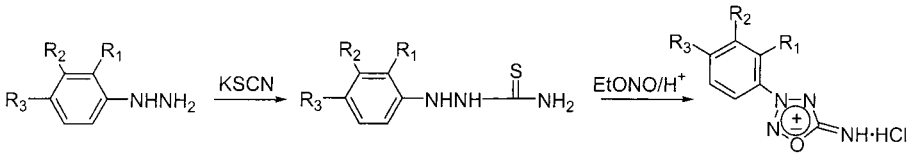
Figure 25.

typical compounds: GEA 3162 (1,2,3,4-oxatriazolium-5-amino-3-(3,4-dichlorophenyl) chloride), GEA 3175 (1,2,3,4-oxatriazolium-3-(3-chloro-2-methylphenyl)-5-[[(4-methylphenyl)sulfonyl]amino-]-, hydroxide inner salt), GEA 5024 (1,2,3,4-oxatriazolium-5-amino-3-(3-chloro-2-methylphenyl) chloride), and GEA 5624 (*N*-5-[1,2,3,4-oxatriazolium-3-(3-chloro-2-methylphenyl)]-*N*′-3-propynyl carbamate).

1. Synthesis

Initially, the 3-aryl-1,2,3,4-oxatriazole-5-imines were synthesized from monohydrazines via cyanohydrazines and nitrosocyanohydrazines, which then could be converted into acyl derivatives by treatment with various acylating agents.[510] An alternative synthetic route was developed by Karup et al.[512,513] As shown in Scheme 37, reaction of hydrazines with potassium thiocyanate under acidic conditions gives the corresponding 1-arylthiosemicarbazides, which are nitrosated in acidic ethanol with ethyl nitrite to give the 3-aryl-1,2,3,4-oxatriazole-5-imines as a hydrochloride salt.

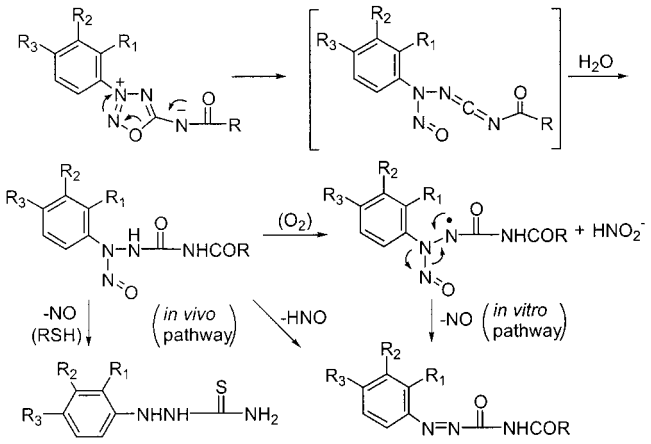
Scheme 37



2. Reactions

The hydrochloride salts of 3-aryl-1,2,3,4-oxatriazole-5-imines have been known to be stable for as long as 5 years or more. NO release from these compounds strongly depends on their concentrations, temperature, pH, and most importantly chemical structures. The optimal pH for NO release is a range of pH 6.2−6.8, close to the pK_a value of the salts. The 5-imino-substituted 3-aryl-1,2,3,4-oxatriazole-5-imines, such as amides, sulfonamides, and urea types, are slower NO releasers. In vitro, one may approximate the release of NO in the following order: imines > amides = sulfonamides > ureas. A proposed NO release mechanism is shown in Scheme 38. The in

Scheme 38



vivo and in vitro pathways differ from each other. Enzymatic degradation or the presence of thiols may increase the release of NO in vivo.[514]

3. Biological Applications

The biological activities of several typical oxatriazole-5-imines have been extensively investigated. They are potent antiplatelet, fibrinolytic, thrombolytic, and broncholytic agents.[515,516] Intracerebroventricular administration of GEA 3162 induces short-lasting and dose-dependent hypotensive response without significant changes in the heart rate, while sodium nitroprussde and SIN-1 increase the heart rate without affecting blood pressure.[517] In addition, GEA 3162 has a protective effect on isolated rat heart in ischaemia and reperfusion.[518] GEA 3162 has also been shown to cause dramatic and concentration-dependent induction of apoptosis.[519] *N*-Formyl-methionyl-leucyl-phenylalanine-induced neutrophil activation is inhibited by GEA 3162. A low dose of the intragastrically administrated GEA 3162 inhibits gastric ulceration induced by ethanol in anaesthetized rats.[520] When administrated intravenously, the GEA 3162 has no effect on ethanol-induced gastric lesions, although a clear blood pressure lowering effect can be seen. GEA 3162 inhibits endothelial cell-mediated oxidation of low-density lipoprotein.[521] Cuthberton et al. reported that GEA-3162 increased stimulated lipopolysaccharide-mediated interleukin-8 (IL-8) accumulation from neutrophils as well as increasing interleukin-8 accumulation in unstimulated cells.[522] In contrast, Lahteenmaki et al. reported that GEA-3162 did not affect IL-8 accumulation but did cause an increase in elasterase accumulation.[523,524] GEA-3162 has been shown to inhibit the suppression of apoptosis in lipopolysaccharide-stimulated polymorphonuclear leukocytes.[525]

GEA 3175, which is more potent than SIN-1 and SNAP in inducing relaxations of bovine bronchioles,[526] elicits relaxations through a cyclic GMP-dependent mechanism followed by opening of large conductance Ca^{2+}-activated K^+ channels. GEA 3175 also inhibits lipopolysaccharide-induced adhesion.[527,528] The increased E-selectin expression induced by lipopolysaccharide was significantly attenuated by the NO donor, whereas intercellular adhesion molecule-1 expression remained unaltered.

GEA 5024 shows a strong antimalaria activity.[529] In addition, it causes a dose-dependent apoptotic cell death in both metastatic and nonmetastatic K-1753 murine melanoma cells.[530] GEA 5024 induces dose-dependent inhibition of maximal insulin-stimulated glucose transport in both muscles with minor effects on basal uptake values.[531] Microinjection of GEA 5024 into rostral ventromedial medulla of naïve animal dose-dependently facilitated the tail-flick reflex.[532]

L. Sydnonimines

Sydnonimines, mesoionic heterocycles, are an important class of NO donors.[533−535] Figure 26 il-

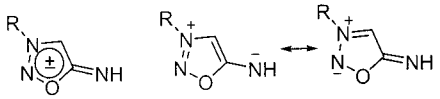
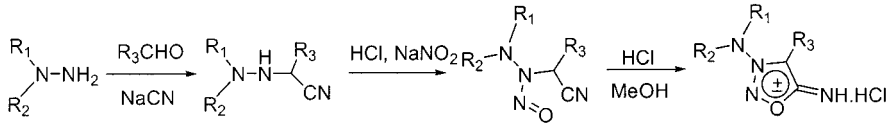


Figure 26.

lustrates their general structures and resonances. This type of compound was first synthesized independently by Brooke et al.[536] and Ohta et al.[537] in 1957.

Molsidomine (*N*-ethoxycarbonyl-3-morpholinosydnonimine), SIN-1 (3-morpholinosydnonimine), CAS 936 (3-(*cis*-2,6-dimethylpiperidino)-*N*-(4-mehoxybenzoyl)-sydnonimine, pirsidomine), C87−3754 (3-(*cis*-2,6-dimethylpiperidino)-sydnonimine, linsidomine), C4144(3-(3,3-dimethyl-1,4-thiazane-4-yl)sydnonimine hydrochloride), and C89−4095 (3-(3,3-dimethyl-1,1-dioxo-1,4-thiazane-4-yl)sydnonimine hydro-

Scheme 39



chloride) are the most extensively studied in this class; their structures are shown in Figure 27.

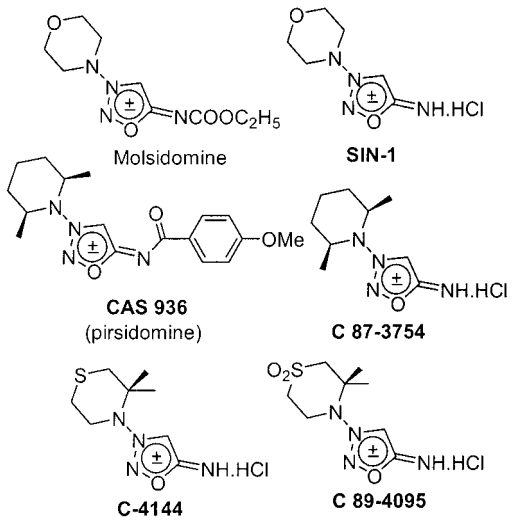


Figure 27.

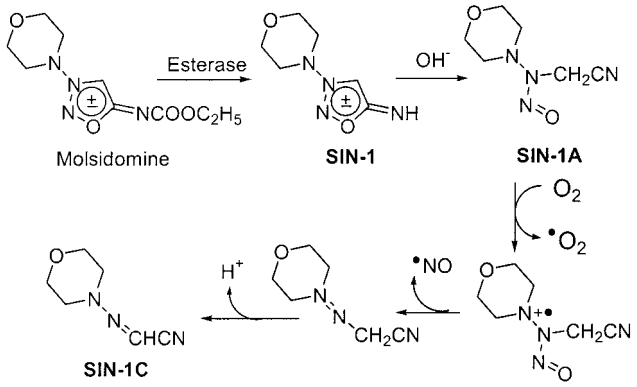
1. Synthesis

The synthesis of 3-diakylaminosydnonimines and their *N*-acyl derivatives was reported by Masuda et al.[538,539] As shown in the Scheme 39, the 1,1-dialkyl-2-α-cyanoalkylhydrazines are obtained by α-cyanoalkylation of 1,1-diakylhydrazines. Subsequent nitrosation leads to the unstable intermediate, 1,1-diakyl-2-nitroso-2-α-cyanoalkylhydrazine, which will cyclize to 3-dialkylaminosydnonimines and can be isolated as a monohydrochloride by the addition of excess hydrochloric acid. The *N*-acyl derivatives can be synthesized by various acylating methods.[540,541] The chemistry of sydnonimine has been extensively reviewed by Yashuskii and Kholodov.[542]

2. Physical Properties and Reactions

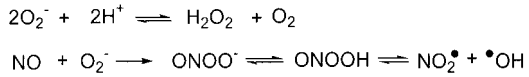
Molsidomine and related *N*-acyl derivatives of sydnonimines are stable solids which can be stored, protected from light, at room temperature. Molsidomine can be decomposed by successive enzymatic and nonenzymatic steps. As shown in the Scheme 40, it can be deacetylated in the liver to yield SIN-1.[543] At physiological and more alkaline pH, SIN-1 undergoes rapid nonenzymatic hydrolysis to form the ring-opened product, SIN-1A. Under strictly anaerobic conditions SIN-1A is stable at pH 7.4, provided the solution is protected from light. Traces of oxygen promote oxidative conversion to a cation radical intermediate which, upon NO release and deprotonation, undergoes spontaneous cleavage to the corresponding *N*-morpholino-aminoacetonitrile, SIN-1C.[544] Furthermore, irradiation with visible light can remarkably enhance the oxygen-dependent NO release from SIN-1.[545]

Scheme 40



In the course of this reaction stoichiometric amounts of superoxide anions ($^{\bullet}O_2^-$) are formed as a result of oxygen reduction. In addition, H^+ ions are generated as well. Since NO is known to react with $^{\bullet}O_2^-$ at an almost diffusion-controlled rate,[546] peroxynitrite ($OONO^-$) production is inevitable. The decomposition of peroxynitrite leads to the formation of highly reactive hydroxyl radical ($^{\bullet}OH$) which may initiate lipid peroxidation in human low-density lipoprotein (Scheme 41).[547,548] Significantly, the manifestation of

Scheme 41



biological activity of sydnonimines is accompanied by oxygen uptake. A close correlation has been found between the O_2 uptake and NO release. The decomposition of sydnonimines generates a superoxide anion; the relative rates of oxygen uptake and O_2^- generation directly correlated. NO release from SIN-1A, however, is independent of oxygen. Oxidants other than O_2, and certain redox-active enzymes, can promote oxidation and NO release from the corresponding *N*-nitrosohydrazine forms of sydnonimines. For example, SIN-1 can be oxidized by cytochrome *c* in a superoxide-independent manner under anaerobic conditions.[549,550]

3. Biological Applications

Sydnonimines were initially used as antihypertensive agents.[551,552] Molsidomine has been used in several European countries as an antianginal drug.[553] In animal and human studies, molsidomine brings about the reduction of the venous return, cardiac output, ventricular work, and myocardial oxygen consumption.[554−557] One of the main advantages of molsidomine is the absence of tolerance. Whereas molsidomine itself is only poorly vasoactive in vitro, its metabolite SIN-1 is a potent vasorelaxant and antiplatelet agent. These activities are mediated largely by spontaneous release of NO. Soluble gua-

nylyl cyclase activation by sydnonimines is independent of the presence of thiols and can differ by a factor of 1000 among different compounds of this class.[558,559] In addition, SIN-1 kills *Escherichia coli* in direct proportion to its concentration with an LD_{50} of 0.5 mM. The bactericidal activity of SIN-1 is further enhanced by pterin plus xanthine oxidase. Hydrogen peroxide was not directly toxic to the bacteria, but *E. coli* pretreated with hydrogen peroxide was more susceptible to SIN-1.[560]

CAS 936 is a syndonimine derivative similar to molsidomine with prolonged vasodilating action in vitro[561] and prolonged hypotensive effects following oral application in dogs.[30,31] In vitro, CAS 936 relaxes endothelium-denuded guinea pig pulmonary arteries contracted with potassium-induced depolarization, probably by a mechanism not related to NO formation.[562] However, CAS 936 in vivo acts similarly to other nitrovasodilators, based on NO release and activation of soluble guanylyl cyclase.[563] CAS 936 appears to be subject to enzymatic degradation. Its metabolite C87−3754 induces endothelium-independent dilation of noradrenaline-contracted rabbit aorta and femoral arteries.[564] In addition, C-4144, a sydnonimine hydrochloride, showed a promising pharmacological profile due to its balanced properties regarding photolytic and thermal stability and kinetics.[565] Sydnonimine derivative C89−4095 was found to exert NO-dependent antithrombotic properties in vivo due to the inhibition of platelets.[566]

M. Oximes

Alkyl- and aryloximes can be converted to NO under oxidative conditions. Some of these compounds such as NOR-1, NOR-3, and NOR-4 (Figure 28) have

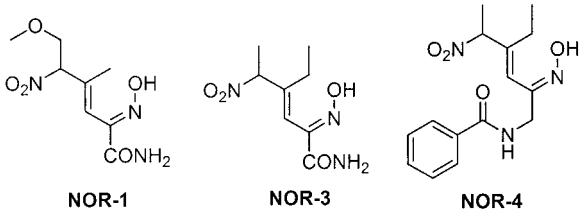


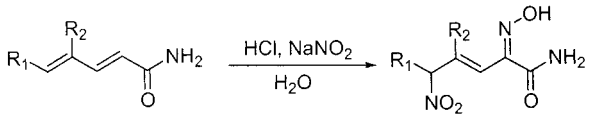
Figure 28.

been shown to be vasoactive, with activity related to NO.[567] Up to now, these NO donors have been commercially available and widely used in biological studies. However, the most well-studied compound is NOR-3.

1. Synthesis

The synthesis of this type NO donor is achieved by a key "nitro-nitrosation" step, which introduces a nitro group and a hydroxyimino group into the diene moiety of the molecule in one step (Scheme 42).[568,569]

Scheme 42

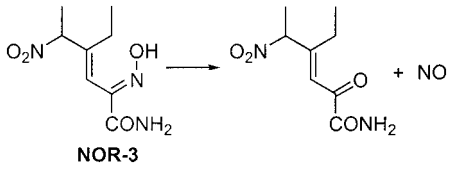


The "nitro-nitrosation" is conducted by dropwise addition of hydrochloric acid to an aqueous solution of the substrate and $NaNO_2$. Oximes can be prepared from their corresponding amines in biological systems. For example, some aliphatic primary amines were efficiently *N*-oxygenated in the presence of liver microsomes to hydroxylamines and then to oximes mainly by the flavin-containing monooxygenase.[570] NOR-3 can also be obtained from the fermentation broth of *Streptomyces griseosporeus* strain.[571]

2. Physical Properties

NOR-3 is stable as a solid when stored at low temperature. In aqueous solution, at pH 7.4, it decomposes to the corresponding ketone derivative and NO (Scheme 43).[572] The half-life is around 45

Scheme 43



min. Structural modifications of NOR-3 lead to compounds with distinct half-lives and NO-releasing properties.[573,574] For example, NOR-4 was shown to be twice as stable in aqueous solution as NOR-3 and to release NO at about one-half its rate.[575] Methoxy substitution at the α-carbon atom leads to compound NOR-1, which decomposes in minutes.

Koikov et al. reported that some oximes of 2-arylmethylene- (**66**) and 2-arylmethylquinuclidi-3-one (**67**) (Figure 29) produced NO under mild oxida-

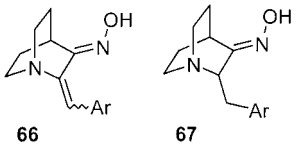


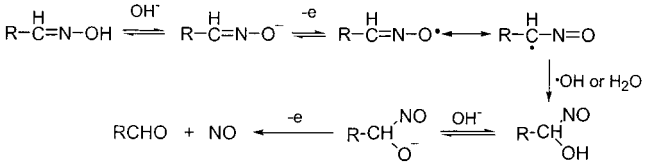
Figure 29.

tion.[576,577] The most active NO donors containing a 2-OH-phenyl group proximal to the oxime fragment could activate soluble guanylate cyclase. 4-OH-isomers, 2-MeO- and 4-MeO-derivatives, as well as the oxime of quinuclidin-3-one were proved to be significantly less active.

3. Reactions

The mechanism leading to NO generation from these oximes has been proposed.[577] At pH 12, all the oximes are ionized >90%; therefore, the first step in the oxidation is undoubtedly the loss of an electron from their anion (Scheme 44). The resultant radical

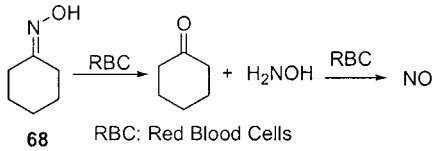
Scheme 44



reacts with hydroxy radical or water to give a *C*-nitroso-*C*-hydroxy intermediate. Further ionization and loss of an electron lead to NO release.

Another possible mechanism was suggested by using cyclohexanone oxime (**68**) as the model, which is a hematotoxic compound.[578−580] The in vivo metabolism of **68** was found to release NO.[581] Incubation of **68** with venous blood resulted in the formation of the characteristic nitrosylhemoglobin complex, suggesting that the blood was a possible site for metabolism. Hydroxylamine, a compound with strong oxidative capacity with regard to hemoglobin, was suggested to be the intermediate in the metabolism (Scheme 45).[581] The observed toxicity of **68** may be

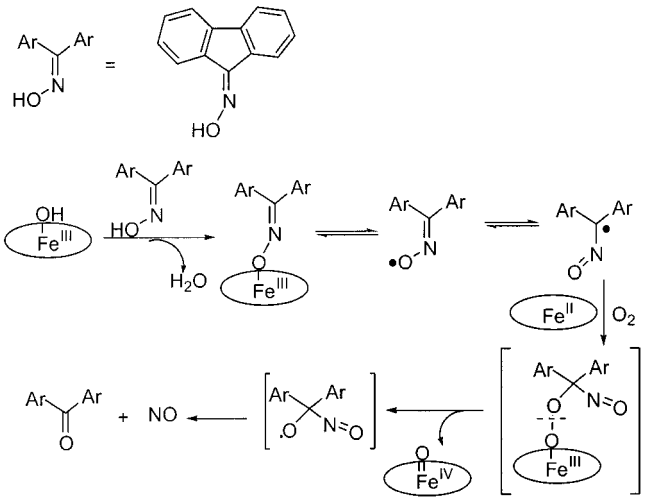
Scheme 45



due to the excessive NO production.

The oxidation of oximes by O_2 forming NO can be catalyzed by an iron porphyrin.[582] As shown by Groves and co-workers (Scheme 46), oxime reacted

Scheme 46



first with hydroxoiron(III) porphyrin (Fe(OH)P) to generate a five-coordinate, high-spin oximatoiron(III) porphyrin species [Fe(oximate)P]. Then, the reaction proceeded via an Fe−O bond homolysis followed by O_2 insertion to generate 9-nitroso-9-fluorenylperoxyFe(III)TMP, which decomposed via an O−O bond homolysis to generate NO, ketone, and oxo-Fe(IV)P.

4. Biomedical Application

Although many oximes have the potential to release NO, very few of them like NOR-type compounds have been used in biological studies as NO donors. They are structurally unique vasodilators which produce a potent vasorelaxation in isolated dog coronary arteries and the rat aorta.[583−585] About 50% of the applied dose of NOR-3 was recovered as nitrite/nitrate in the urine.[585] Reports show that antiplatelet effects of NOR-3 are more potent than those of organic nitrates such as isosorbide dinitrate,[586,587] based on the potential of spontaneous NO generation. Recently, the protective effect of NOR-3 on ischemic acute renal failure (ARF) in rats has been demonstrated,[588] suggesting that the spontaneous NO donors might be clinically effective in cases of ischemic ARF.

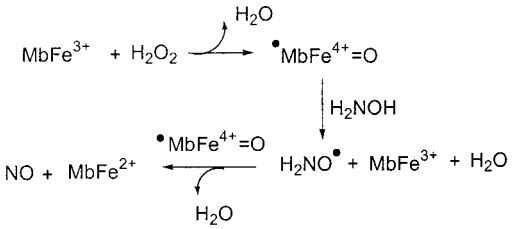
N. Hydroxylamines

Hydroxylamine (HA) is a natural product of mammalian cells.[589−592] The free base of HA is unstable and very hygroscopic. However, the hydrochloride or sulfate salts of HA are crystalline, water-soluble compounds which can be stored for years at room temperature. In strongly alkaline aqueous solution, HA is known to slowly disproportionate to NH_3, N_2, and N_2O, the latter presumably via intermediate formation of nitroxyl.

Enzymatic production of NO from HA by catalase was reported many years ago.[593,594] Formation of the NO−catalase complex was evident by ESR spectroscopy in test solutions containing HA, catalase, and a glucose−glucose oxidase, H_2O_2-generating system. Nonenzymatic attack of hydroxylamine by superoxide anion can also account for the production of NO.[595] Since superoxide anion is produced in many tissues, especially inflammatory cells, a reaction between superoxide anion and HA is possible. This nonenzymatic pathway would lead to the formation of NO and other derivatives such as peroxynitrite.

Taira et al. found that NO could be generated from HA in the presence of myoglobin (Mb) and H_2O_2.[596] As shown in Scheme 47, $MbFe^{3+}$ is first oxidized by

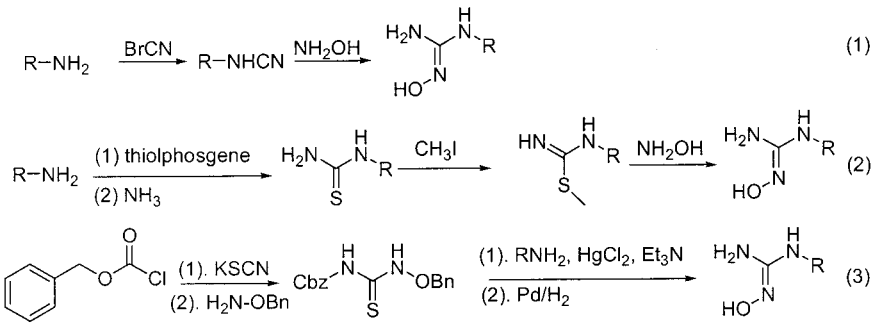
Scheme 47



H_2O_2, producing FerrylMb ($^{\bullet}MbFe^{4+}{=}O$), a two-electron oxidation product. FerrylMb is a potent oxidizing agent which oxidizes HA (or its derivatives) to a nitroxide radical. Then the nitroxide radical reacts with FerrylMb to release NO. Interestingly, although all three hydroxylamines (HA, *N*-methyl HA, and *N*-dimethyl HA) have been found to yield corresponding nitroxide radicals, only HA was able to produce free, diffusible NO.

HA is widely used as an NO donor and exhibits a wide range of biological activities.[597−600] For example, HA causes dose-dependent vasodilation in the blood vessels of rat kidneys.[601] HA can relax endothelium-denuded rat aortic rings and rabbit aortic strips in a dose-dependent manner.[602−604] HA can also inhibit insulin release and activate K^+ channels.[605−608]

Scheme 48



O. *N*-Hydroxyguanidines

Hydroxyguanidines, combining the imino group of guanidine with hydroxylamino group of hydroxyurea, have been shown to exhibit cytotoxicity in human leukemic cells and antitumor activity in vivo.[609−612] NO production from hydroxyguanidines was suggested to be the mechanistic basis for the cytostatic and cytotoxic effects.[613] Unlike other type of NO donors, *N*-hydroxyguanidines do not generate NO spontaneously. It needs the catalysis of enzyme such as nitric oxide synthases (NOS) and P450. Several hydroxyguanidines have been demonstrated to be substrates of NOS (Figure 30). Among these compounds, *N*-hydroxy-L-arginine (NHA) is the natural intermediate in the in vivo enzymatic conversion of L-arginine to NO, upon NOS stimulation. It displays vasorelaxant and cytostatic activity, which may, in part, be endothelium-dependent and/or caused by direct chemical interaction of the compound with released NO.

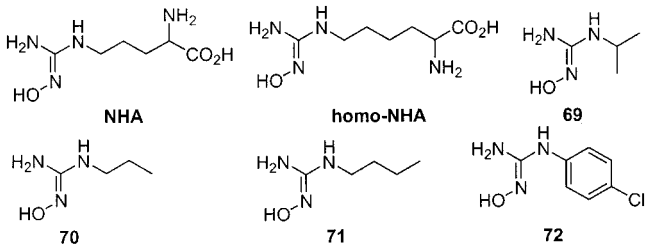


Figure 30.

1. Synthesis

There are three major procedures for the synthesis of the hydroxyguanidine moiety (Scheme 48). The most widely used method involves a cyanamide as the key intermediate. Cyanamide can be prepared from reacting an amine with BrCN. It is further reacted with hydroxylamine to produce hydroxyguanidine.[614−617] The second method utilizes isothiourea as a crucial intermediate, which has been used in the synthesis of NHA.[618] Recently, a convenient reagent, 1-benzyloxy-3-benzyloxycarbonylthiourea, was developed for the introduction of the *N*-hydroxyguanidine moiety.[619,620] The Cbz group was chosen for *N*-protection since the removal of both Cbz and Bn groups could be accomplished simultaneously under mild conditions.

2. Physical Properties

N-Hydroxyguanidines are stable compounds when kept at −20 °C. They are unstable with respect to base. Typically they are isolated as the salt of a strong acid. Because of the guanidine function, L-arginine is the most basic of all amino acids. *N*-Hydroxylation of the guanidine function, however, significantly decreases its basicity (the pK_a of arginine is 13.6 and the pK_a of NHA is 8.1).[621] In fact, the structure of NHA has been depicted by various authors as the oxime tautomer **73**, the imine tautomer **74**, and protonated species **75** (Figure 31).

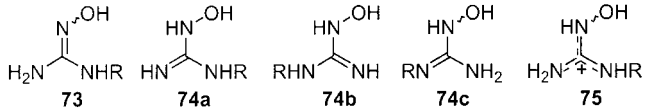


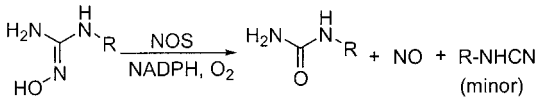
Figure 31.

Recently, based on a combination of theory and experiment (ENDOR and X-ray), it was concluded that structure **75** is the most likely form of NHA bound to NOS.[622,623] In addition, *N*-radical formation is shown to be a plausible alternative to *O*-radical formation in the NOS-catalyzed conversion of **75** to L-citrulline and NO.

3. Reactions

In the NO biosynthesis, it is the guanidine fragment of L-arginine that undergoes oxidation to the NHA and further to citrulline and NO by NOS. The binding of the substrates with NOS is largely due to the guanidine fragment (with the heme of the enzyme and a special 'guanidine binding' site).[624−628] It could be expected that some compounds with guanidine/hydroxyguanidine or similar structures could act as substrate for NOS and be transformed via the oxidative NO−synthase mechanism with the evolution of NO. However, very few guanidine-containing compounds have been clearly shown to be the substrates of NOS. Besides endogenous NHA, *N*-hydroxy-L-homoarginine, *N*-aryl-*N*′-hydroxyguanidines, and some *N*-alkyl-*N*′-hydroxyguanidines have been demonstrated to be substrates (Figure 30).[629−633] The mechanism of the oxidation of these exogenous compounds by NOS is similar to NHA. The major products are ureas and NO, with the cyanamide as a minor second metabolite (Scheme 49). The very limited number of substrates for NOS suggests that NOS requires highly specific structural features. Recent structural

Scheme 49



study reveals that there is an important nonpolar van der Waals interaction between substrate (or inhibitor) and NOS.[634] Crystal structural studies of NOS oxygenase domains indicate a small hydrophobic cavity formed by two conserved amino acid residues (Phe and Val) in close proximity to the catalytic site. The distance between this cavity and *N*-methylene in NHA is around 5 Å (roughly equivalent to the length of *n*-propyl group). Thus, with attachment of suitable hydrophobic group to the hydroxyguanidine functional group, could yield a suitable substrate for NOS.

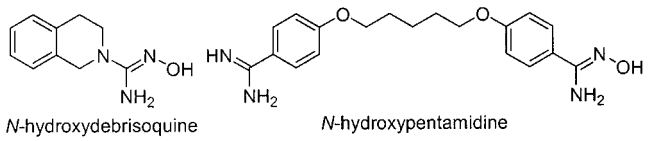


Figure 32.

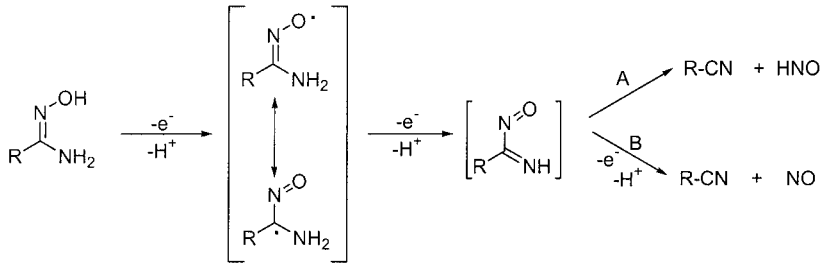
Unlike NOS, cytochrome P450s can oxidize most *N*-hydroxyguanidines to generate nitrogen oxides ($NO/NO_2^-/NO_3^-$). For instance, *N*-hydroxydebrisoquine is transformed into its corresponding urea by rabbit liver microsomes or by purified P450 2C3.[635−637] Cytochrome P450 2C3 also catalyzes the *N*-hydroxylation of one of the amidine functions of the drug pentamidine, with formation of *N*-hydroxy-pentamidine (Figure 32). This metabolite is further oxidized to the corresponding amide and nitrogen oxides, including NO.[638] The oxidation of *N*-hydroxyguanidines by P450s not only leads to the corresponding ureas but also to the corresponding cyanamides. Similarly, oxidation of amidoximes leads to the corresponding amides and nitriles.

The oxidative cleavage of C═N bonds to the corresponding C═O bonds involves a P450-dependent transfer of one oxygen atom from O_2 to the substrate, with concomitant formation of nitrogen oxides. The reaction mechanism has been proposed by Mansuy and co-workers (Scheme 50).[639,640] It involves a one-electron oxidation of hydroxyguanidines or amidoximes with formation of an intermediate iminoxy radical. The second step is the one-electron oxidation of this radical leading to nitrosoimine. Elimination of HNO from the nitrosoimine directly produces RCN and HNO (path A), whereas one-electron oxidation of nitrosoimine leads to RCN and NO (path B).

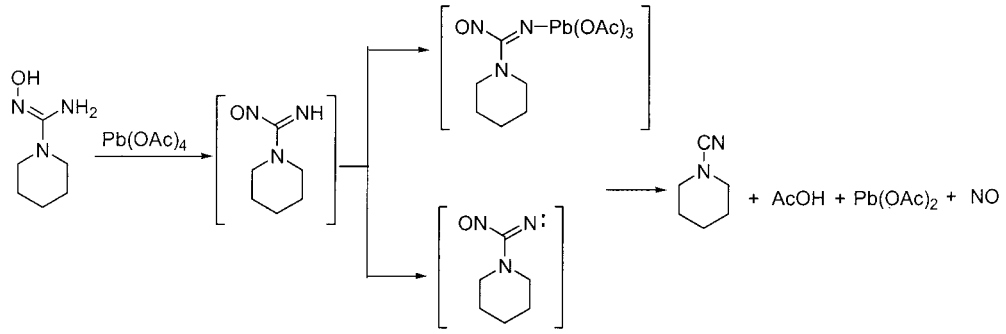
The chemical oxidation of *N*-hydroxyguanidines, such as NHA, *N*-butyl-*N*′-hydroxyguaindine, and *N*-(*N*-hydroxyamidino)piperidine (NHAP), has been studied by Fukuto and co-workers.[641,642] Different oxidants can give different products. Lead tetraacetate and potassium ferricyanide/hydrogen peroxide produce NO (Scheme 51). Other oxidants such as Fe(III), PbO_2, Ag_2CO_3, and peracids produce HNO as the product. This is not really unexpected since HNO can be predicted as the two-electron oxidation product, whereas NO requires an overall three-electron oxidation, and the majority of the chemical oxidants utilized in previous studies are two-electron oxidants. Also, it has been demonstrated that peroxidation of NHA by NOS results in the generation of HNO.[643] The organic product from the oxidation of *N*-hydroxyguanidines can be either the urea, the citrulline equivalent product, or the cyanamide, depending on the oxidant. In 1996, Ishikawa and co-workers reported that photosensitized oxygenation of hydroxyguanidines led to the effective production of the expected urea derivative along with generation of NO or its equivalents.[644] The formation of both products could be explained by the mechanism based on a singlet oxygen ene reaction on olefins.

Recently, a chemical model for the H_2O_2-promoted oxidation of *N*-hydroxyguanidines by NOS has been

Scheme 50



Scheme 51



developed.[645] Biomimetic oxidation was carried out using H_2O_2 and tetrakis(perfluorophenyl)porphyrinatoiron(III) chloride ($FeTPPF_{20}$) as a catalyst. Similar to NOS, this system produces cyanoornithine, citrulline, and NO from NHA.

4. Biological Applications

The bioactivity of hydroxyguanidine compounds has been observed for a long time. In 1973, this class of compounds was shown to be an antihypertensive agent.[646] Recently, novel *N*-hydroxyguanidine derivative PR5 (1-(3,4-dimethoxy-2-chlorobenzylideneamino)-3-hydroxyguanidine) has been shown to be an alternative electron acceptor in xanthine oxidas-catalyzed oxidation of xanthine.[647] This finding suggests that it belongs to a novel class of drugs with xanthine oxidase electron-acceptor/blocking properties that might be used as protective agents in ischemia−reperfusion. Furthermore, Nitromed has claimed NHA and other *N*-hydroxyguanidines, optionally with coadministration of other vasoactive agents, as a novel method for vasodilation and treatment of diseases such as pulmonary, sexual dysfunction, hypoxia, cardiovascular disorders, and even loss of memory. The ability of NHA to produce relaxation of rabbit corpus cavernosum and rat aortic segments was demonstrated, whereas arginine was not effective in these experiments. The effects on aortic segments from diabetic rats were more pronounced compared to nondiabetic models.[648]

P. Hydroxyurea

As a derivative of hydroxylamine, the pharmacology of hydroxyurea (Figure 33) has also greatly

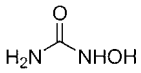


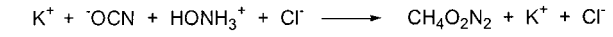
Figure 33.

drawn scientists' attention.

Actually, biomedical application of hydroxyurea possesses a long history in human beings with promising therapeutic effects. It was originally investigated as an antitumor agent.[649] In limited cases of cervical[650] and uterine[651] cancer, hydroxyurea was found to be effective without a significant impact on cellular mechanisms. It has been suggested as a radiosensitizer in patients with carcinoma of the cervix and in head or neck cancer.[652] Nowadays, hydroxyurea has become standard therapy for chronic myelogenous leukemia, polycythemia vera, and myeloproliferative disorders.[653] It is considered as an effective systemic agent for treatment of severe psoriasis,[654] a persistent skin disease caused by an abnormality in the functioning of key white cells in the blood stream triggering skin inflammation. As an antiretroviral agent, hydroxyurea was found to block ribonucleotide reductase and result in inhibition of proviral DNA synthesis.[655,656] This provides the basis to study the inhibition of human immunodeficiency virus (HIV) replication by hydroxyurea, making it a possible candidate for AIDS therapy.[657] In fact, recently it has been prescribed by physicians to treat HIV, though much less frequently than standard antiretrovirals. A double-blinded, randomized clinical trial using hydroxyurea as a treatment for sickle cell disease showed that it could reduce the number of painful crises in some patients.[658,659] Nowadays, hydroxyurea represents a new treatment for sickle cell anemia.

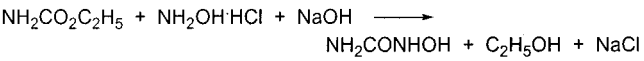
Hydroxyurea has been synthesized from potassium cyanate and hydroxylamine hydrochloride by Dresler and Stein in 1860s (Scheme 52).[660]

Scheme 52



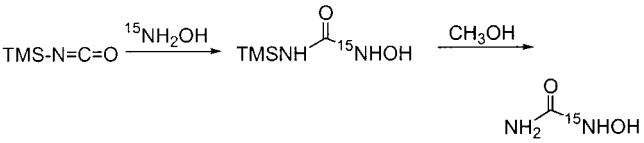
About 30 years later, Francesconi and Parrozzani obtained a lower melting isomeric substance by letting solid potassium cyanate and hydroxylammonium chloride react in test tubes at low temperature.[661] On the basis of the former literature[662] and their own preliminary experiments, Kofod assumed that the unsatisfactory yields are due to a pronounced lability of the isomers or of some intermediates in aqueous solution at higher temperature. He conducted and worked up the reaction at low temperature, and the two isomers of hydroxyurea, with the corresponding melting point 71 and 140 °C, were isolated by extracting with boiling anhydrous ether and absolute ethanol successively.[663] The structural problem of hydroxyurea isomers has aroused lots of discussions.[664] Runti and Deghenghi came up with another procedure to synthesize the higher melting isomer in 1953,[665] and it was adapted and published in *Organic Syntheses* 20 years later (Scheme 53).[666]

Scheme 53



As an essential tool for determining the source of biologically relevant nitrogen monoxides from hydroxyurea, Yasaki et al. recently reported a simple and efficient synthesis of ^{15}N-hydroxyurea from commercially available starting materials as shown in Scheme 54.[667]

Scheme 54



Hydroxyurea decomposes very rapidly in aqueous acidic medium, whereas its metallic salts (sodium or the copper complex salts) are stable. It is preferable to store the crystals in a cool, dry place. Some decomposition may occur after a few weeks. Hydroxyurea is readily absorbed after oral administration, reaches peak blood levels in 2−4 h, and is excreted in the urine with a half-life of less than 8 h.[668] It enters cells by passive diffusion and is distributed throughout body water.

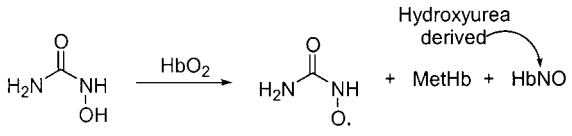
Structural comparison with urea suggests that the diverse biological roles of hydroxyurea can be attributed to the hydroxylated nitrogen atom adjacent to the ketone. Resembling to hydroxylamine or *N*-

hydroxyguanidine, this NO unit structure gave rise to a serious question: Does hydroxyurea take effect via an NO mechanism? Chemically, the treatment of hydroxyurea with hydrogen peroxide and copper(II) sulfate produces a "NO-like" species capable of N-nitrosating morpholine.[669] Sato et al. investigated the NO generation by H_2O_2-dependent peroxidation of hydroxyurea in the presence of copper-containing proteins such as Cu, Zn-superoxide dismutase (Cu, Zn-SOD) or ceruloplasmin as a catalyst, and the result indicated that NO release from hydroxyurea might be mediated by •OH derived from the copper-catalyzed Fenton-like reaction.[670] Both hydroxyurea and NO inhibit ribonucleotide reductase and quench the catalytically essential tyrosyl radical of M2 subunit of this enzyme.[671,672] Hydroxyurea has also been found to decompose to NO in the presence of peroxidase[673] and hemoglobin.[674,675] This accumulating result shows that hydroxyurea, just like hydroxylamine and *N*-hydroxyguanidine, is most probably metabolized to NO in vivo and then exhibits its biological effects. Actually, using rat as an animal model, Jiang et al. first observed NO generation in vivo after administration of hydroxyurea.[676]

The detailed molecular mechanism of hydroxyurea in treatment of sickle cell anemia remains poorly described, and its clinical efficacy has mainly been attributed to the increased level of fetal hemoglobin (HbF), which reduces the tendency of HbS, a mutant form of hemoglobin, to polymerize.[658,659,677] However, some patients benefit from hydroxyurea treatment before their HbF levels increase, indicating that the positive effects of this drug cannot be completely explained by HbF level increase and suggesting other mechanisms of action.[678] Previous explanations include a proposal that hydroxyurea increases mean cell volume (MCV) and reduces neutrophil count.[659]

In the past few years, the reaction between hydroxyurea and oxyhemoglobin (HbO_2) to form methemoglobin (metHb) and nitrosyl hemoglobin (HbNO) has been taken into account, as HbNO plays an important role in blood pressure control.[679] In 1998, Xu et al. reported for the first time that in vitro reaction of sickle cell oxyhemoglobin with hydroxyurea to form sickle cell nitrosylhemoglobin involved the specific transfer of NO from the NHOH group of hydroxyurea (Scheme 55).[681]

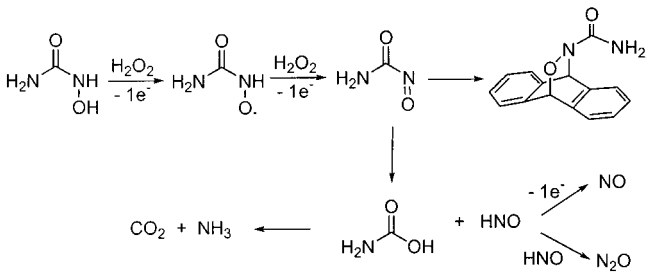
Scheme 55



Evidence that the NO group of HbNO derived from the NHOH group of hydroxyurea was obtained by the use of ^{15}N-labeled hydroxyurea and the observance of an isotope effect on the hyperfine splitting pattern of the EPR spectrum of HbNO.

Model oxidation of hydroxyurea with hydrogen peroxide followed by product analysis indicated that hydroxyurea decomposed with the formation of nitroxyl (HNO) and NO (Scheme 56).

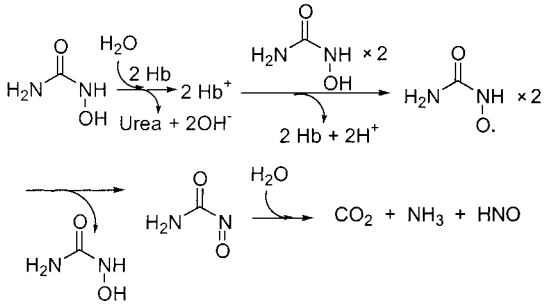
Scheme 56



The presence of nitroxide radical was also detected by EPR spectroscopy. Nitrous oxide identification provided strong evidence for the intermediacy of HNO, which rapidly dimerized and dehydrated to form nitrous oxide (N_2O) as the major nitrogen oxide. Additional evidence relevant to the mechanism of hydroxyurea oxidation included the trapping of a *C*-nitrosoformamide intermediate, which resulted from the cycloaddition reaction between 9,10-dimethylanthracene (9,10-DMA) and the intermediate *C*-nitrosoformamide.

Further investigation into the conversion of hydroxyurea showed that the unligated form of ferrous hemoglobin, deoxyhemoglobin, also reacted with hydroxyurea; in addition, the overall reaction proceeded much faster than that to oxyhemoglobin.[682] The same products as those arising from the reaction of hydroxyurea with oxyhemoglobin had been detected and confirmed by isotope-labeled technology and EPR spectra; however, their formation in this reaction involving deoxyhemoglobin cannot be explained via the same mechanism. Comparing it with the reaction between hydroxylamine and deoxyhemoglobin,[683] Kim-Shapiro et al. proposed a mechanism as shown in Scheme 57.[684]

Scheme 57



The kinetics of the reaction of hydroxyurea with myoglobin (Mb), hemin, HbS, and normal adult hemoglobin were determined using optical spectroscopy as a function of time, wavelength, and temperature. Each reaction appeared to follow pseudo-first-order kinetics. The results suggested that any heme-containing protein may be capable of oxidizing hydroxyurea with the formation of NO adducts.[685]

It is noteworthy that evidence of NO production from hydroxyurea metabolism in human has been demonstrated when Glover et al. reported that oral administration of hydroxyurea for the treatment of sickle cell anemia produced detectable nitrosyl hemoglobin.[686]

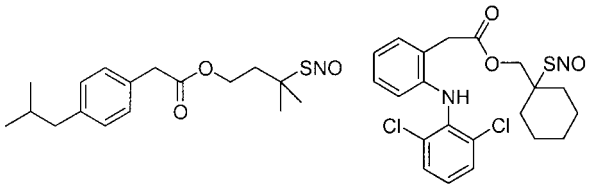
III. Latest Trends in the Development of NO Donors

A. NO Donor/Drug Hybrids

A recent innovative development in NO donor drug research is the hybridization of NO donor moieties with currently available drugs.[29,687] Adding an NO-releasing group to another well-established drug molecule might overcome or reduce the drug's toxicity as well as provide an additional NO-dependent biological activity.

1. RSNO/Drug Hybrids

NitroMed has attached an *S*-NO moiety as the NO donor functionality to ibuprofen (**76**), diclofenac (**77**), and other nonsteroidal antiinflammatory drugs (NSAIDs) (Figure 34). All these derivatives acted as



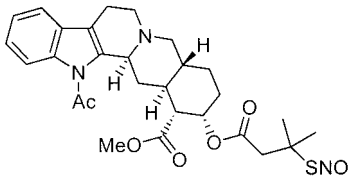
76 77

Figure 34.

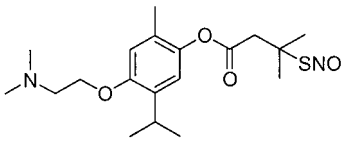
orally bioavailable prodrugs. They were gastric-sparing in that they elicited markedly fewer stomach lesions as compared to the stomach lesions caused by a high equimolar dose of diclofenac in rats.[688] The nitrosothiol esters of diclofenac comprise a novel class of NO-donating compounds having therapeutic potential as nonsteroidal antiinflammatory agents with an enhanced gastric safety profile.

NitroMed also attached an *S*-NO moiety with other bioactive molecules such as steroid and dopamine agonists (Figure 35).[689−694] Hybrid **78**, when compared with fluticasone, was shown to be a more effective inhibitor of the immediate response in the ascaris-sensitive sheep assay, which makes **78** and other similar compounds attractive in the treatment of asthma and other respiratory disorders. Compound **79** was compared with dipyramidole for its ability to relax the phenylephrine-induced contraction of human corpus cavernosum tissue biopsy samples and proved to be superior by about 50%.

Nitrosylated α-adrenergic receptor antagonists (α-ARAs), a novel type of bifunctional NO releasing drugs, were also designed and evaluated as potential agents for the treatment of impotence.[695] Compared with their parent compounds, nitrosylated yohimbine (NMI-187) and moxisylyte (NMI-221) (Figure 36),



NMI-187



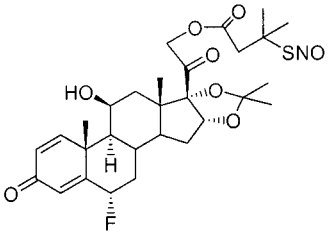
NMI-221

Figure 36.

which have bifunctionalities of an NO donor and an α-ARA, are more potent. More importantly, only the nitrosylated compounds can induce the accumulation of cyclic GMP in rabbit corpus cavernosum strips. These compounds might have therapeutic value for the local pharmacological treatment of impotence.

2. Nitrate/Drug Hybrids

Nicox, another company involved in extensive drug discovery and development on therapeutic NO modulation, has designed several patented nitroxylated NSAID prodrugs, most of which are nitrate hybrids. These compounds include aspirin/nitrate hybrids NCX-4016 [(3-nitrooxymethyl)phenyl (2-acetoxy)benzoate] and NCX-4215 [(3-nitrooxy)butyl (2-acetoxy)-benzoate).[696−699] The former possesses a second benzene ring to which the lateral chain containing the NO group is bound.[700] These compounds were originally designed to protect the gastric mucosa against the effects of aspirin. NO generating compounds have been shown to reduce the severity of mucosal injury, probably due to NO and prostacyclin production. Prostacyclin protects gastric mucosa by preserving blood flow and increasing the synthesis of mucus.[701,702] NO aspirins possess antiaggregating effects because of inhibition of cyclooxygenase (COX) by aspirin and formation of soluble guanylate cyclase by NO.[703] NCX4016 also appears to retain the antithrombotic and antiinflammatory actions of the COX inhibitor with the added benefit of protection against ulceration of the gastric mucosa.[704−707] Besides NO−aspirin hybrids, NO−mesalamine (NCX-456) has



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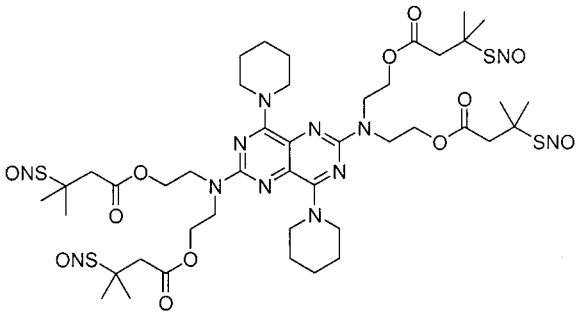

79

Figure 35.

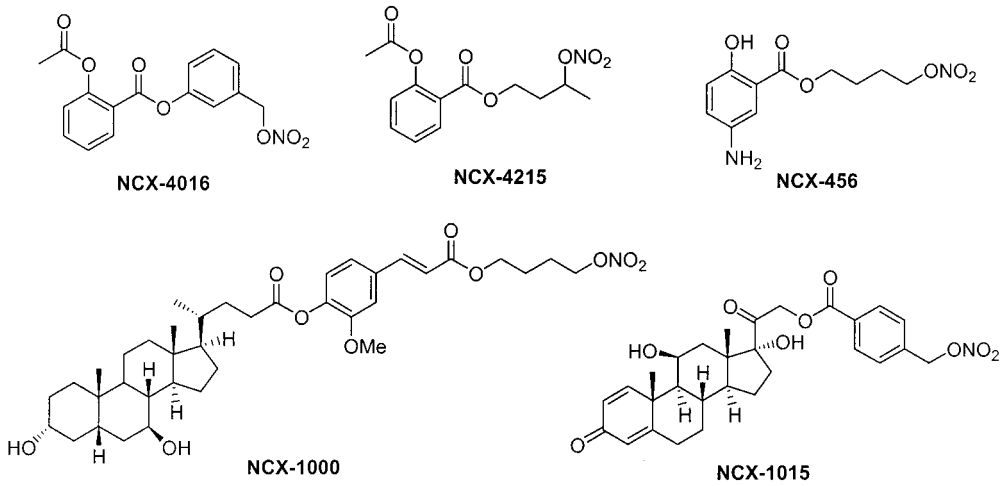

Figure 37.

enhanced antiinflammatory effects and can inhibit effector caspases and protect colonic epithelial cells from cytokine-induced apoptosis.[708,709] NCX-1000 and NCX-1015 (a novel NO/prednisolone derivative) are steroid/NO hybrids. The antiinflammatory activity of NCX-1015 was enhanced with the attachment of an NO donor.[710,711] The addition of an NO-releasing moiety to ursodeoxycholic acid (NCX-1000) confers effective immunoregulatory and antiapoptotic activities to this compound. These effects are caused mainly by the *S*-nitrosation/inhibition of both the proapoptotic and proinflammatory branches of the caspase superfamily, Figure 37.[712]

CAL International designed an ISMO− acetylsalicyclic acid derivative **81** to treat cardiovascular disorders.[713] Other *O*- and *S*-nitrooxyacylated salicylic and thiosalicylic acid derivatives such as **82** have been introduced as new types of nitrate/salicylate and nitrate/thiosalicylate hybrids.[714] NycoMed designed compound **83** in which a nitrite is attached to a chelating carrier.[715] This compound is reported to be comparable to most classic organic nitrates without any significant resistance. Furthermore, it is claimed that the compound is useful in reducing the cardiotoxicity of antitumor drugs, reperfusion injury, retroviral infections, and inflammation. In another study, Lehmann and co-workers synthesized nitrate/dihydropyridine hybrids. Those compounds combined an NO donor either with calcium channel blocker (predominantly arteriodilating, negative inotropic, **84a**) or with calcium channel activator (vasoconstrictive, positive inotropic, **84b**); Figure 38.[716−717]

In view of the ample evidence supporting the involvement of superoxide in the mediation of tolerance to organic nitrates and cross-tolerance to other NO donors, Haj-Yehia et al. hypothesized that incorporation of an antioxidant group within an organic NO donor might not only prevent tolerance development, but also give an added or even synergistic vasodilation effect. To evaluate this hypothesis, they developed novel organic nitrates, such as 3-nitratomethyl-PROXYL (**85**) and TEMPO-4-mononitrate (**86**) (Figure 39),[718,719] that, in addition to being a classical

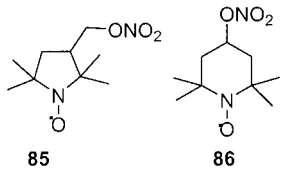

Figure 39.

NO-donor, also possesses a potent anti-superoxide (SOD-mimic) action. For example, compound **85** is the first compound that can simultaneously and favorably affect both NO and superoxide. This simultaneous bifunctionality may underlie the potent vasodilatory action of **85** without induction of tolerance. Since the ratio between NO and superoxide constitutes a major determinant of cellular function, bifunctional agents such as **85** may prove useful in the pharmacotherapeutic management of a long series of oxidative stress-mediated pathologies in which an imbalance between NO and superoxide exists.

3. Furoxan/Drug Hybrids

To obtain new vasodilators capable of displaying both NO-dependent effects and the α_1-antagonist activities, furoxan analogues of Prazosin were syn-

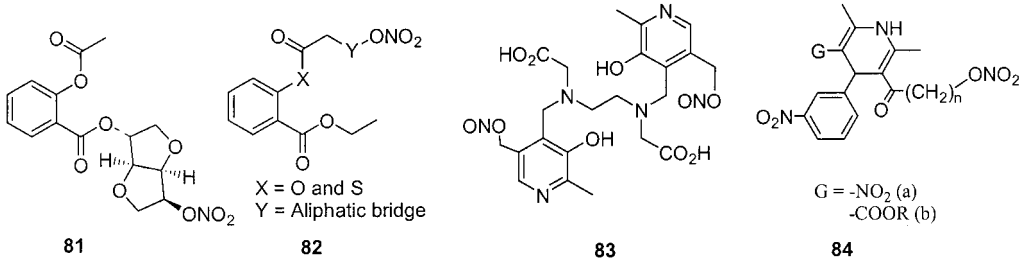

Figure 38.

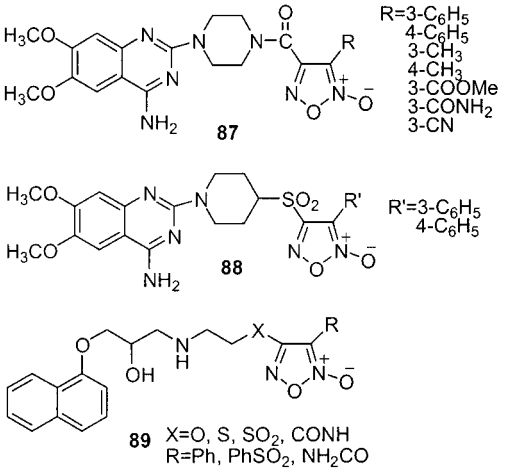

Figure 40.

thesized in which the phenyl (or methyl) furoxanylcarbonyl system was substituted for a 2-furanylcarbonyl moiety.[720] The resulting hybrids (**87** and **88**) displayed various pharmacological behaviors. When the 4-furoxanylcarbonyl system, bearing an ester or an amide function at the 3-position, was present, hybrids with predominant α_1^- antagonist activity were obtained. In contrast, in the derivative in which the nitrile function is linked to the 3-position of the furoxan ring, an NO-mediated vasodilation effect is predominant. Furoxansulfonyl piperidine derivatives showed both NO vasodilation and α_1-antagonist activities in an appropriate balance. The hybrids with mixed NO-dependent vasodilator and β-blocking activities (**89**) have also been reported.[721,722] The β_1- and β_2-blocking activities were examined on isolated guinea pig right atria and on guinea pig trachea, respectively. Vasodilation was assessed on endothelium-denuded strips of rat aorta. Some derivatives behaved as well balanced "hybrids" displaying both NO-dependent vasodilating and β-blocking properties in the same concentration range. Some others displayed either prevalent β-blocking or vasodilatory activity.

Di Stilo et al. prepared a series of 4-phenyl-1,4-dihydropyridines (**90**−**93**) substituting at the ortho and meta positions of the phenyl ring with an NO-donating furoxan moiety (Figure 41).[723] These new

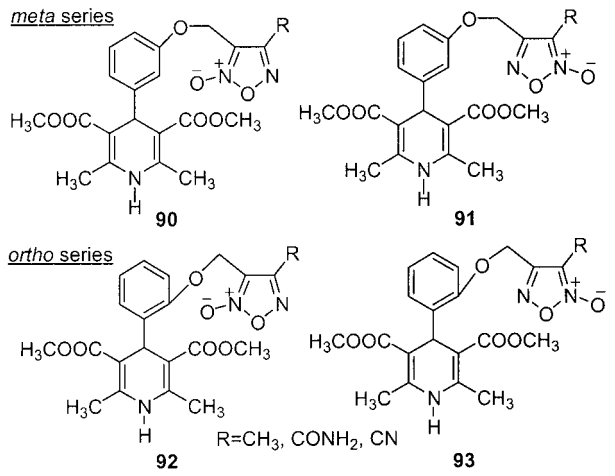

Figure 41.

1,4-dihydropyridiens conjugated furoxanyl moieties possess both nitric oxide-like and calcium channel antagonist vasodilator activities.

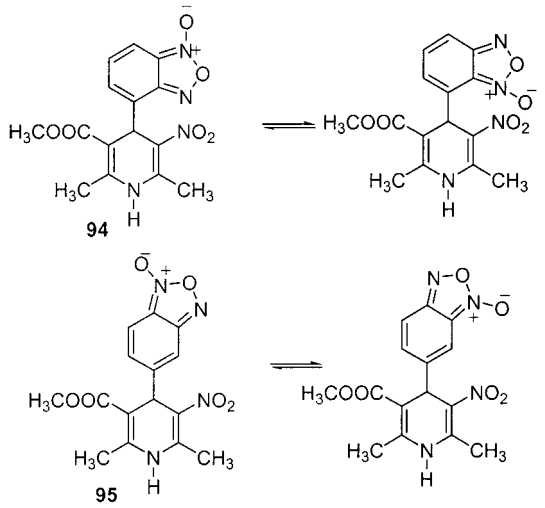

Figure 42.

Visentin et al. reported the synthesis and voltage-clamp studies of methyl 1,4-dihydro-2,6-dimethyl-5-nitro-4-(benzofuroxanyl)pyridine-3-caboxylates (**94**, **95**) (Figure 42).[724] The racemic mixture was resolved into the corresponding enantiomers. Whole-cell voltage-clamp studies on L-type Ca^{2+} channels expressed in a rat insulinoma cell line showed that all the dextrorotatory antipodes were effective agonists of L-type Ca^{2+} currents, while the levorotatory ones were weak Ca^{2+} entry blockers.

Very recently, Mu et al. prepared a series of hybrid molecules (**96**−**98**) incorporating the furoxan and nicorandil moieties with cardiovascular and cerebrovascular activities (Figure 43).[725] The compounds

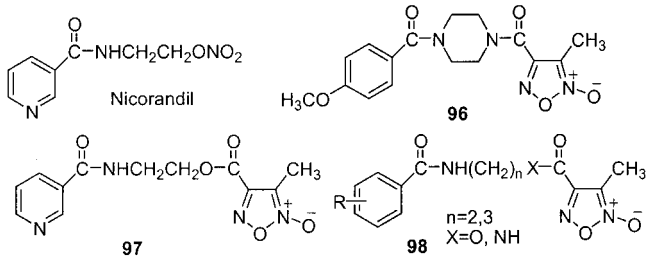

Figure 43.

were tested on KCl-induced contraction of rabbit thoracic aorta whose endothelium was denuded. Compound **96** exhibited a gradual and sustained hypotensive effect on anaesthetised rats at 1.5 mg/kg.

B. Enzyme-Activated NO Donors

Although many different potent NO donors have been found and used in biological studies, the inexorability of NO release can be a major disadvantage in many biomedical applications, particularly in the design of drugs for remedying disorders resulting from local deficiencies of endogenous NO. Accordingly, the ultimate goal of the NO-related drug discovery effort is to devise strategies for selectively

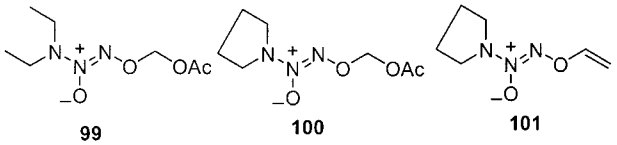

Figure 44.

delivering NO to the sites of need while avoiding exposing other NO-sensitive parts of the body to its multipotent effects.[726] One of the strategies is to convert these spontaneous NO-releasing agents to stable prodrugs by attaching them to carriers, which could be specially recognized by certain targets such as enzymes. If one could choose a carrier that would be selectively removed in the target organ or cell type of interest, dosage with NO could be concentrated at that site even if the prodrugs were systemically distributed. On the basis of the above idea, Keefer and co-workers developed a novel NO prodrug class which attached their diazeniumdiolate family to a $-CH_2OAc$ group.[727] These compounds (**99**, **100**) are stable in neutral aqueous media but release NO upon metabolism by esterase. They appear to offer substantial cell-targeting advantages over the NONOate anions from which they were derived. The observed increase in leukemia cell antiproliferative potency (up to 2 orders of magnitude or more relative to the corresponding anions) suggests considerable potential utility for intracellular targeting of NO in a variety of applications. Prodrug **101** was designed as an organ-selective NO donor to protect the liver from apoptotic cell death.[728] It increased liver cGMP levels while minimally affecting systemic hemodynamics, protecting rats dosed with tumor necrosis factor-α plus galactosamine from apoptosis and hepatotoxicity. Wang's group developed several NO prodrugs using Cupferron and diazeniumdiolate as NO-generating moieties. One example is the attachment of Cupferron to *N*-acetylphenylalanine through *O*-alkylation resulting in *N*-(*N*′-acetylphenylalanylmethylenyloxy)-*N*-phenyldiimide *N*-oxide (**102**).[281] This compound was essentially stable at neutral pH. Only very slow NO release was observed. However, upon treating with α-chymotrypsin, NO release was significantly accelerated. In another report, they coupled diazeniumdiolate to the terminal carboxyl groups of a series of amino acids and peptides.[729] The peptide sequences were chosen as prostate-specific antigen (PSA) substrates. Prodrugs such as **103** could be activated by PSA to release NO. Most recently, they attached diazeniumdiolate functional groups to carbohydrate units, resulting in compounds **104**−**106**.[730] These sugar−NONOates exhibited significantly improved stability as compared to their parent diazeniumdiolate salts while readily releasing NO upon activation by specific glycosidases.

IV. Patented NO Donors

To date, there have been more than 70 U.S. and World patents on the applications of NO donors. Some patented applications for a variety of diseases are briefly summarized below.

A. Cardiovascular Diseases

The fact that NO is produced constitutively by eNOS and acts through the guanylate cyclase-cGMP pathway is critical to the maintenance of blood-vessel homeostasis, blood pressure, and organ perfusion. There is strong evidence that the bioactivity of endothelium-derived NO is impaired in atherosclerosis and related disease states. NO donors can be used in either acute or preventive treatment of myocardial ischemia or acute heart failure.[731−734] Some NO donors are used as vasodilators.[735−739] NO donors also show antiplatelet activity.[740−742] Other usages include treating blood platelet disorders[743] and increasing the permeability of the blood brain barrier.[744] There are some new NO donors, such as oxadiazolopyrimidine oxides,[745] pyridyl-1,2,5-oxadiazolecarboxamide 2-oxides,[746] NO-metal complexes,[747] *N*-oxy-*N*-nitrosamines,[748] and NO-releasing polymers,[749,750] which are useful in treating cardiovascular diseases.

B. Central Nervous System Diseases

In the central nervous system, NO acts as intracellular and extracellular messengers. Depending on the cellular compartment, the level of NO produced, or the setting in which it is produced, NO may be toxic or protective to the brain in ischemic condition. While the production of NO from either nNOS or iNOS leads to neurotoxicity, NO production from eNOS protects brain tissue by maintaining regional cerebral blood flow. It was also found that NO donors promote the secretion of neurotrophic factors from mammalian central nerve cells. Thus, some NO donors have been patented for the treatment of nerve degeneration-associated diseases.[751,752]

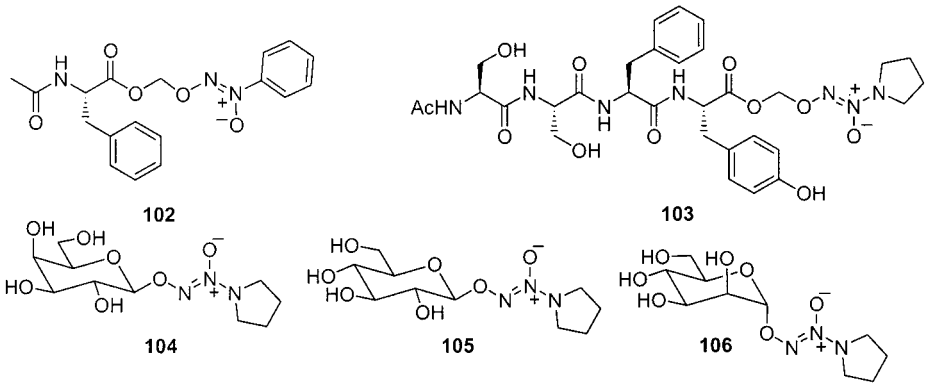

Figure 45.

C. Diseases Related To Immunity

iNOS-derived NO is an important modulator in the immune system. It mediates both normal tissue function and tissue injury. Thus, NO donors can act as protective agents during inflammation.[99,753−757] These effects may be related to actions of NO on several targets including prevention of leukocyte−endothelial adherence, maintenance of mucosal blood flow, and stimulation of mucus secretion. In addition, NO also has antimicrobial[758,759] and antitumor activities.[760,761]

D. Physiological Disorders

It has been shown that NO reduced bone resorption of osteoclasts via inhibition of cysteine protease cathepsin K, which is believed to be a key protease in bone resorption.[762−764] NO donors can also be used in climacteric disorders and menstrual disorders.[765−769] Since the physiological mechanism of erection of the penis involves release of NO during sexual stimulation, some NO donors have been used in the treatment of sexual dysfunction.[690,770,771] NO donors also have beneficial effects for many other biological disorders.[719,772−780]

E. Other Usages

Besides almost all types of major diseases, NO donors are proposed to be useful in many other medical settings. One example is that they can be used on the surface of medical devices.[781−787] Many medical devices suffer from surface adhesion of blood platelets. To minimize this thrombogenic effect, blood thinners such as heparin, coumarin, and aspirin are often used. However, systemic administration of those antiplatelet agents concomitantly increases the risk of uncontrolled bleeding elsewhere in the body. In contrast, NO-generating polymer-coated devices can prevent this potentially life-threatening side effect, since NO is a potent inhibitor of platelet adhesion, aggregation, and activation. Some usages include making kidney perfusion solutions,[788] delivery of medicant to the brain,[789,790] treatment of airway constriction,[791] inducing hair growth,[792] as well as others.[793−796] NO donors can also be used in animal diseases, i.e., gut spasm,[797] and laminitis in horses.[798]

V. Conclusion

In the past decade, hundreds of NO donors have been developed and widly used in biological research. The current development of NO donors includes discovery of new NO donors, finding novel applications for NO donors, development of NO−drug hybrids, and development of NO donors with good tissue specificity. Among these four fields, the development of NO donors with good tissue specificity is the most challenging work for chemists and biochemists. The problem is related to the ubiquity of NO itself in the body. Since NO has so many roles, it is difficult to devise an NO donor that affects only one physiological function. However, people have realized and tried to solve the problem. For example, attaching NO donors to tissue-specific ligands, in particular monoclonal antibodies, may result in target selectivity. Furthermore, in many situations, such as postcoronary angioplasty, it will be possible to deliver these drugs locally where they are needed and in this way to obviate undesired side effects. NO donors have kept chemists intrigued for more than 10 years, and there are more good years ahead.

VI. Acknowledgment

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VII. References

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