Exogenous donors of nitric oxide (a chemical aspect)

V. G. Granik and N. B. Grigor´ev*

Federal State Unitary Enterprise
State Scientific Center "Scientific Research Institute of Organic Intermediates and Dyes,"
1, building 4, ul. Bol´shaya Sadovaya, 103787 Moscow, Russian Federation.
Fax: +7 (095) 254 9724. E-mail: makar-cl@ropnet.ru

The review considers problems related to the formation, in the living organism, of nitric oxide, a versatile and vitally important regulator of cell metabolism. The pathways of formation of endogenous nitric oxide from L-arginine are discussed and the main approaches to increasing the NO concentration by introducing various types of exogenous nitric oxide donors into the organism and chemical and biological characteristics of these donors are considered. Primary attention is devoted to the known drugs that were shown to release NO under hydrolytic, oxidative, or reductive conditions. The solution of problems related to the elucidation of the mechanisms of drug action requires that the formation of nitric oxide be taken into account.

Key words: nitric oxide, nitric oxide release, NO-synthases, L-arginine, exogenous nitric oxide donors, soluble guanylate cyclase, pharmacology, drugs.

Introduction

During the last 15 years, the views on cell metabolism regulators have dramatically changed. It was found that a low-molecular-mass compound, *viz.*, nitric oxide NO, is a versatile and necessary regulator of cell metabolism. It is surprising that a gas and, moreover, a toxic gas whose molecule is a short-lived species that readily enters into diverse chemical transformations is constantly formed in the mammalian organism under the action of enzymes and affects various physiological and pathophysiological processes. Nitric oxide participates in the regulation of the blood-vessel tone, inhibits the platelet aggregation and platelet adhesion on the blood vessel walls, and operates in the central and vegetative nervous systems by regulating functions of the respiratory apparatus, the gastrointestinal tract, and the urogenital system. Nitric oxide exhibits a fairly broad range of biological activities. It comes as no surprise that back in 1992, the editors of the *Science* journal called NO the molecule of the year, and in 1998, three scientists from the USA, R. F. Furchgott, L. J. Ignarro, and F. Murad, were awarded the Nobel Prize in physiology and medicine for contribution to the elucidation of the role of nitric oxide in the functions of a living organism. The number of original publications and reviews devoted to the problem of NO grows like an avalanche every year.[1—12] The purpose of this review is to summarize the most important data concerning NO donors that appeared during the last five years (after publication of our previous review[4]). In addition, we would like to draw the attention of researchers to the necessity of more extensive use of chemical and physicochemical concepts for interpreting the complex phenomena involved in the synthesis and functioning of NO in the organism. Combination of biological and chemical approaches is expected to provide the basis for better understanding of the observed phenomena, more rigorous conclusions, and better predictability for the most promising lines of research in this field of science.

The first section of the review discusses general problems related to the functions of nitric oxide in the organism; then enzymatic synthesis of nitric oxide from arginine is considered. This is followed by consideration of the effect of NO on soluble guanylate cyclase, which is the main target for this regulator in the body. Data on exogenous donors of nitic oxide are considered in detail, the attention being focused on the problems and prospects of investigations along this line in the search for new drugs.

1. General aspects

Nitric oxide is a colorless gas soluble in water (1.9 mmol L^{-1} at 25 °C), which is readily oxidized by atmospheric oxygen in aqueous media. In aqueous solutions in the presence of oxygen, nitric oxide is mainly converted into nitrite anion because it is only partly

Published in Russian in *Izvestiya Akademii Nauk. Seriya Khimicheskaya,* No. 8, pp. 1268—1313, August, 2002.

oxidized to NO_2, while the subsequent transformations proceed as follows:[12]

$$NO + 0.5\ O_2 \longrightarrow NO_2, \quad (1)$$

$$2\ NO_2 \rightleftharpoons N_2O_4, \quad (2)$$

$$NO + NO_2 \rightleftharpoons N_2O_3, \quad (3)$$

$$N_2O_3 + H_2O \longrightarrow 2\ NO_2^- + 2\ H^+, \quad (4)$$

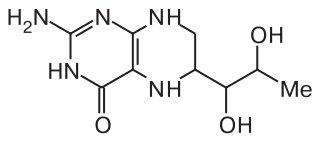
$$N_2O_4 + H_2O \longrightarrow NO_2^- + NO_3^- + 2\ H^+. \quad (5)$$

Reactions (3) and (4) are much faster than reaction (5); therefore, the amount of nitrate anions formed is relatively small. Nitric oxide is one of the most simplest molecules with an odd number of electrons and, being a radical, it can react with other compounds and free radicals. The reactions with oxyhemoglobin (to give methemoglobin and nitrate) and with superoxide radical anion $O_2^{\bullet -}$ (to give peroxynitrite anion $ONOO^-$, a strong oxidant, which is also capable of nitrosating proteins and nucleic acids) belong to the most important reactions of NO.

Nitric oxide is an intra- and intercellular regulator and a paracrine (*i.e.*, influencing the functions of neighboring cells) compound. The fact that NO reacts with thiols, proteins, sugars, metal ions, hemoprotein hemes, and other compounds localized in different tissues implies the presence of nitric oxide and/or its complexes in intercellular liquids. Since the NO concentration required to activate soluble guanylate cyclase (sGC) and to increase the cell level of cGMP (see below) is rather low, one may suggest that nitric oxide and its complexes constantly circulate in the blood flow. Probably, none other endogenous compound performs such a versatile role of individual intra- and intercellular regulator in the organism.

2. Enzymatic synthesis of nitric oxide

The formation of NO in the human or animal organism is based on transformation of the guanidine fragment of L-arginine[12,13] under the action of enzymes of the cytochrome P-450-like hemoprotein family, *viz.*, NO-synthases (NOS), involving reduced nicotinamide adenine dinucleotide phosphate (NADPH) as a source of electrons and co-factors, *viz.*, flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), and 5,6,7,8-tetrahydrobiopterin (BH_4).



5,6,7,8-Tetrahydrobiopterin (BH_4)

Two main forms of NO-synthase have been described,[7,8,10] namely, the constitutive NOS (cNOS) and the inducible NOS (iNOS),* located in activated macrophages.** The constitutive isoforms require calcium ions and calmodulin (a protein functioning as a intercellular mediator for calcium ion transfer) for activation and are classified into endothelial (eNOS) and neuronal (nNOS) forms (depending on localization). The former (eNOS) generates NO, which decreases blood pressure and affects platelet aggregation,*** while the latter (nNOS) acts as a neurotransmitter.**** Constitutive NO-synthases are similar to each other; they contain two identical subunits with molecular masses of 130000—150000. Calmodulin is involved in the NOS activation in brain.[8]

The key function of iNOS is the immune protection of the organism, *i.e.*, synthesis of nitric oxide as a cytotoxic and an antiinflammatory agent. The NO formation in the immune system takes place in macrophages. The stimulator cytokines***** are linked to macrophages and trigger a chain of reactions resulting in the synthesis of iNOS in the cell. The resulting iNOS diffuses into an affected cell located in the vicinity of the macrophage where it interferes in the cell metabolism. An important point of action is inhibiting the enzyme aconitase, which catalyzes an early stage in the citric acid cycle (citrate to isocitrate transformation). The nitric oxide formed also participates in the electron transport chain. In addition, NO inhibits the DNA synthesis in a damaged cell.[8,10]

The general pathway of NO biosynthesis[13—17] is presented in Scheme 1.

It is only L-arginine (but not the D-isomer) that undergoes the NOS-induced oxidation to give NO.

The electron transport that determines functioning of the NO-synthases occurs along the chain NADPH → FAD → FMN → Fe^{III} and allows the transformation of arginine into its *N*-hydroxy derivative and

* Constitutive enzymes are the enzymes present in cells in constant amounts, irrespective of the metabolic state of the organism. The concentrations of inducible enzymes in cells vary; normally, they are present in trace amounts; however, in the presence of a substrate, their concentration can increase by more than 3 orders of magnitude.

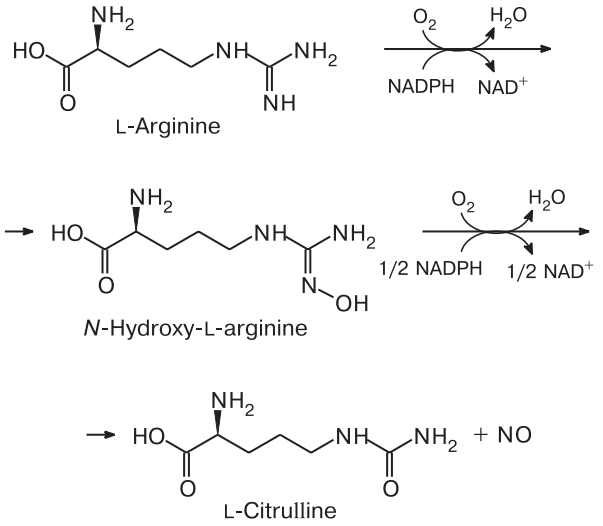
** Macrophages are large cells whose main functions include participation in the natural specific antitumor immunity. Macrophages facilitate the removal of bacteria or other foreign particles from blood and tissues.

*** Platelet is a regular blood element participating in the blood clotting process.

**** Neurotransmitter, which is a low-molecular-mass compound secreted by a neuron ending and bound to a next neuron, serves for transmitting the nerve impulse from a nerve fiber to some cell or muscle fiber or from a receptor cell to a nerve fiber.

***** Cytokines are soluble factors produced by cells for biological action to the nearest cells.

Scheme 1



further into citrulline and nitric oxide. The function of tetrahydrobiopterin is not entirely clear yet. According to an existing opinion, it plays an allosteric rather than a catalytic role, and its presence increases the arginine affinity for the enzyme active site.[17] In the absence of arginine, NOS can produce superoxide radical anion and hydrogen peroxide. In the presence of arginine, the yield of superoxide radical anion is minimized, as this reacts with nitric oxide to give peroxynitrite anion (see below).

It is of note that NO can deactivate NOS by binding as a sixth ligand to iron in the co-factor heme[17,18]

$$Fe^{3+}(heme)NO^{\cdot} \longleftrightarrow Fe^{2+}(heme)NO^{+}.$$

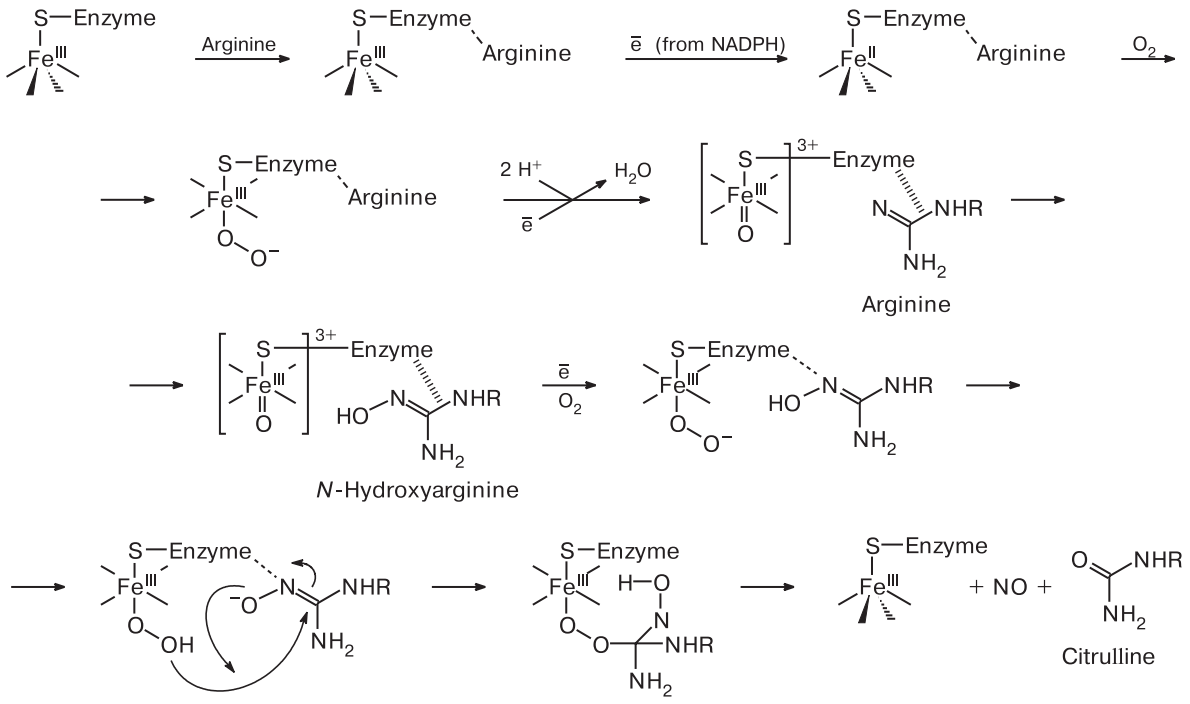
This creates certain problems in the understanding of the mechanism of action of NOS isoforms. Indeed, why does not nitric oxide having a great affinity to the heme cause fast self-inactivation of NO-synthase in this case? This problem has not been solved reliably yet, although some assumptions have been made.

1. At a final stage of the NO and citrulline formation, hydroxy heme arises, prior to the formation of peroxy heme; this loses water rather slowly, so nitric oxide has time to diffuse into tissues and is thus removed from the heme active site.

2. In the oxidation of the key intermediate of the process, *viz.*, *N*-hydroxyarginine, the peroxy heme reacts with the meso-carbon atom of *N*-hydroxyarginine radical anion; in its definite conformation in the enzyme active site, the arising citrulline can be arranged in such a way as to protect the heme from the evolving NO. Dissociation of the NOS—citrulline complex proceeds at a relatively low rate and NO has time to diffuse away from the heme active site.

3. If the ligand is a functional fragment of an amino acid (initial, intermediate, or final), the amino acid can protect the heme from the interaction with NO before the catalytic process starts.[17]

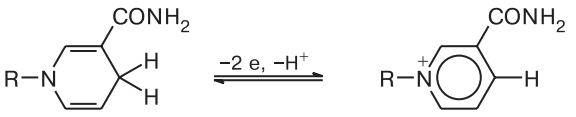
Scheme 2



The fact that NOS contains the heme as a co-factor allows comparison of NOS with cytochrome P-450. On the basis of certain similarity of these systems and the knowledge gained in the studies of P-450, a fairly reliable scheme of the biosynthesis of nitric oxide has been composed (Scheme 2).

The reduced form of nicotinamide adenine dinucleotide (NADPH) is involved in the first step of the electron transport (Scheme 3).

Scheme 3



NADPH fragment

An electron is transmitted from the nicotinamide fragment of NADPH to FAD and then to FMN to give intermediate radicals (Scheme 4).

The transformation of *N*-hydroxyarginine[14,15] to give NO can occur along three pathways (Scheme 5).

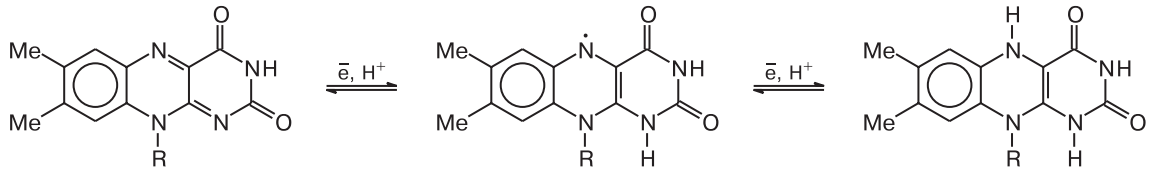
Schemes 2 and 5 do not differ fundamentally and show the pathway of NO biosynthesis from L-arginine, although they require more detailed elaboration.

Despite the extensive investigations performed, many aspects call for more profound studies. The most important question is in what aspects and to what extent the active sites of NOS isoforms differ from one another. The search for selective inhibitors of these enzymes cannot become deliberate without understanding of these aspects.

Apart from the NO-synthase pathway, an alternative route to nitric oxide in the organism has been considered, namely, the nitrite—nitrate—xanthine oxidase route.[19] Xanthine oxidase generates NO from nitrite ions as the substrate and can function in the presence of NADH without oxygen, *i.e.*, the significance of this pathway as a source of nitric oxide increases at hypoxia. According to some data, xanthine oxidase transforms nitrate ions into nitrite ions and then into nitric oxide.

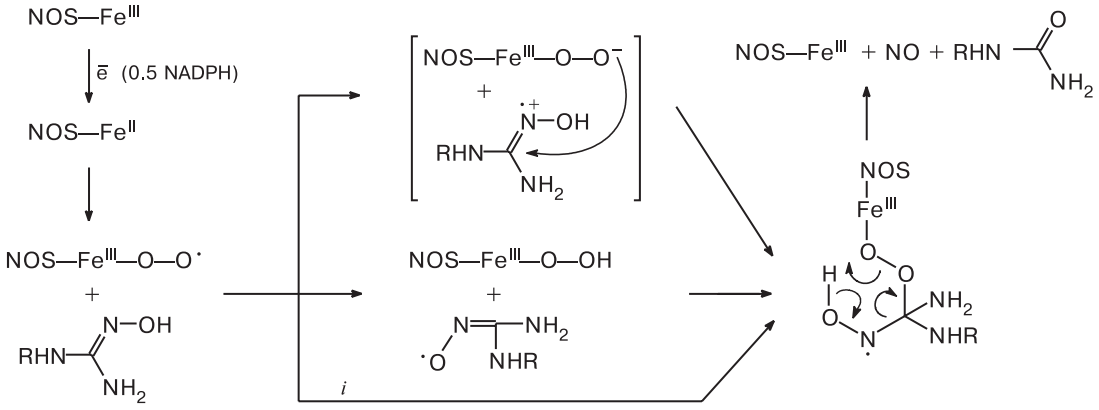
Although the presence of L-arginine as the substrate is important for catalytic oxidation, L-homoarginine and N^G-methyl-L-arginine can serve as alternative substrates. However, these substrates are oxidized less efficiently. Meanwhile, the intermediate *N*-hydroxy-L-arginine is a good substrate for NOS and, apparently, it is not accumulated during oxidation. In our opinion, the search for new compounds able to function as NO donors should not necessarily rely only on NO-synthases. It is the final result, *i.e.*, release of nitric oxide, that is significant but it does not matter whether it is attained in the same way

Scheme 4



A FAD or FMN fragment

Scheme 5

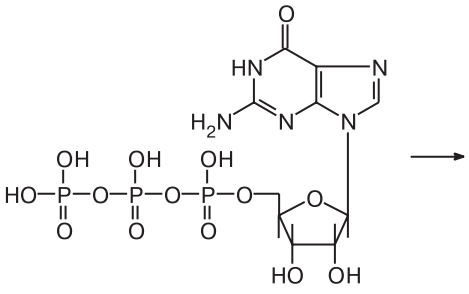


i. Free-radical addition to C=N.

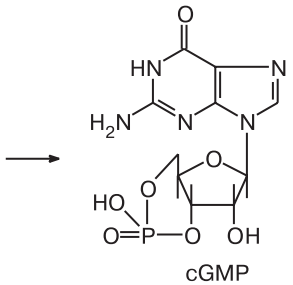
as typical of the NO source in the organism, L-arginine, or involves other enzyme systems.

3. Physiological effects of nitric oxide and soluble guanylate cyclase

The effects of nitric oxide are displayed through interaction with diverse systems of an organism.[1—12] The inducible NOS functions in cells in response to the action of endo- and exotoxins, inflammation mediators, or compounds that activate free-radical oxidation. The induction of iNOS cytokines and the formation of nitric oxide enhance the toxic action of macrophages toward tumor cells, microbes, fungi, and protozoa. Nitric oxide acts as an agent that protects the host organism from foreign bodies. Despite the diversity of biological effects caused by NO release and systems subjected to the action of this metabolic regulator, soluble guanylate cyclase (sGC) localized in the smooth muscles of the vascular wall is the most important physiological target for nitric oxide in the organism. Physiological effects of NO such as its antihypertensive and antiaggregatory action are also related to the functions of guanylate cyclase. Soluble guanylate cyclase catalyzes biosynthesis of cyclic guanosine monophosphate (cGMP) from guanosine triphosphate (GTP).

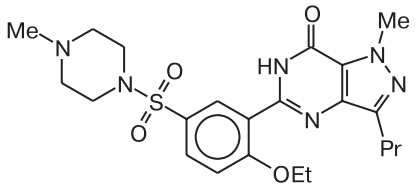


GTP



cGMP

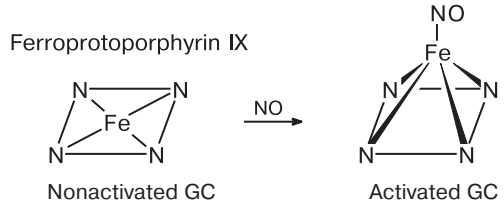
Nitric oxide reacts with the heme, which is the prosthetic group of sGC, to give nitroso heme. The enzymatic activity increases ~50-fold due to the conformational changes similar to those induced by binding of hemoglobin to oxygen. As a consequence, the rate of cGMP production in the neighboring muscle cells increases. Accumulation of cGMP is due not only to sGC activation by exogenous donors of nitric oxide but also to inhibition of phosphodiesterase 5 (PDE 5) responsible for the transformation of active cGMP into inactive GMP. This principle underlies, in particular, the action of viagra, a pyrazolopyrimidine derivative restoring the impaired sexual potency in men.[20]



Viagra

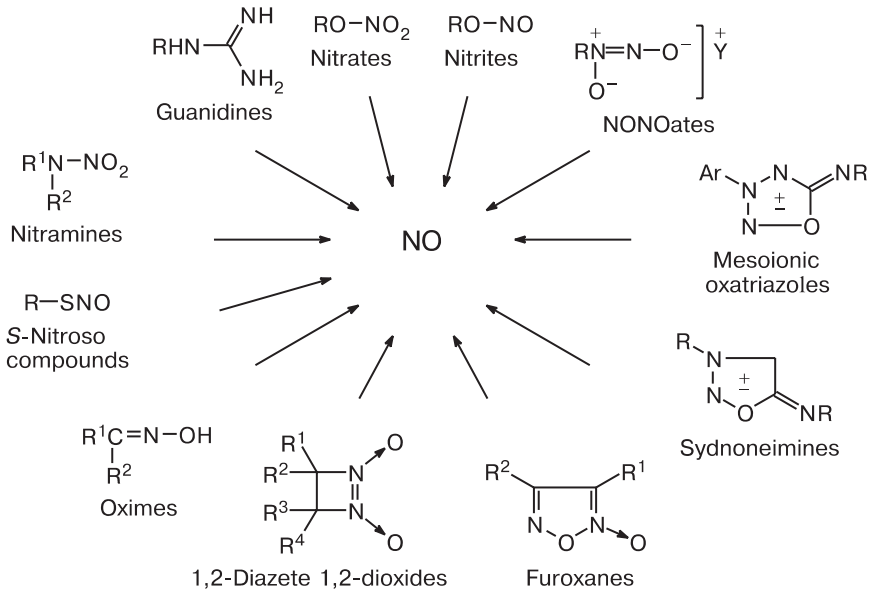
The release and accumulation of cGMP results in the activation of cGMP-dependent protein kinase and Ca^{2+}-ATPase, both of which favor dephosphorylation of myosin light chains and exit of calcium ions from muscle cells; this results in vasodilation and, correspondingly, in the reduction of the blood pressure. Opening of calcium channels generates postsynaptic potentials; this is responsible for the mediator action of cGMP, which functions as the so-called second messenger.* The biological effects are directly related to opening or closure of particular ion channels. A somewhat similar situation occurs upon release of nitric oxide, namely, activation of sGC, which, in turn, favors the formation of cGMP. The latter ensures opening of ion channels and gives rise to a biological effect; in this particular case, this is the antihypertensive effect. The enzyme sGC is characterized by the presence of a heme in the enzyme. Heme-ferroprotoporphyrin IX is formed when iron is incorporated into protoporphyrin IX. Enzyme activation by nitric oxide is believed to be related to the presence of the heme (Scheme 6).

Scheme 6



* This term implies that the mediator, which serves as the primary messenger (evolved from nerve endings to the synaptic gap where it reacts with a postsynaptic receptor to induce a series of biochemical processes), triggers the cGMP formation inside the effector cell; subsequently, cGMP acts as a chemical transmitting agent. This is the chemical transmission mechanism in the organism; as a result, the mediator ensures the occurrence of biochemical processes in the cell without getting into the cell itself.

Scheme 7



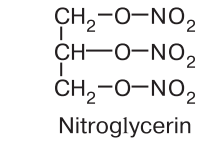
It was found that sGC deprived of the heme loses the susceptibility to NO-induced activation. Yet another problem related to soluble guanylate cyclase is the antiaggregatory activity of NO. It was demonstrated that sGC regulates the aggregation by a feedback mechanism. Indeed, initiation of the platelet aggregation (for example, by addition of ADP) results in the activation of guanylate cyclase and increases the content cGMP, which gives the signal for disaggregation. Evidently, nitric oxide-induced activation of soluble guanylate cyclase is an efficient way of reducing the development of platelet aggregation, leading to thrombus formation. It is important that the regulatory function of sGC is manifested at early stages of aggregation. Therefore, enzyme activators and, hence, nitric oxide or nitric oxide donors can prove to be valuable drugs for the aggregation control and, moreover, for its prevention.

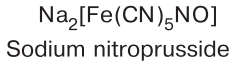
In conclusion, it should be emphasized that study of the action of various materials on the sGC is a convenient trial to verify the ability of substances under testing to release nitric oxide, because the nonheme mechanism of sGC activation is very seldom encountered.

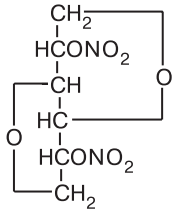
4. Exogenous NO donors

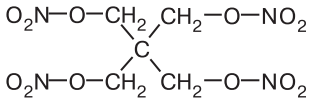
Many pathological states, for example, cardiovascular, infectious, and inflammatory diseases, thromboses, malignant tumors, urogenital diseases, strokes, and so on can be associated with a deficiency or an excess of nitric oxide in the organism. Thus, it is clear that the search for compounds (xenobiotics)* whose transformations in the organism may give rise to NO is a vigorously progressing line of research. Scheme 7 shows the types of NO donors studied previously.[4]

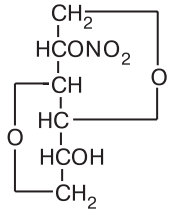
Before we proceed to the results of the most recent studies dealing with the diseases caused by deficiency of NO, it should be emphasized that a number of drugs

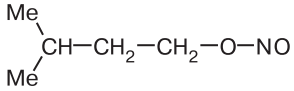

Nitroglycerin

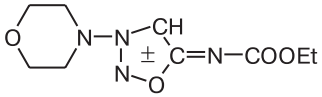

Sodium nitroprusside


Isosorbide dinitrate


Pentaerythritol tetranitrate

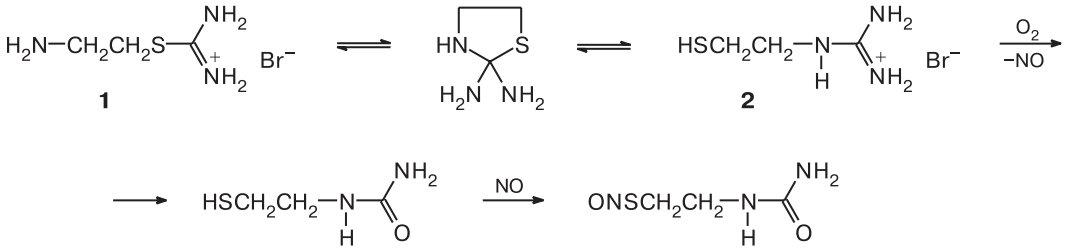

Isosorbide mononitrate


Amyl nitrite


Molsidomine

* Xenobiotics are compounds foreign for the organism.

Scheme 8



whose activity is reasonably attributed to their ability to release nitric oxide in the organism are currently used in medicine. These preparations should actually be classified as prodrugs, because their pharmacological activity is related to the preceding metabolism with nitric oxide release, which is the genuine active principle. These remedies include nitroglycerin, isosorbide dinitrate, isosorbide mononitrate, pentaerythritol tetranitrate, and amyl nitrite; they are antianginal prodrugs (from the Latin *angina pectoris* meaning breast pang) that belong to the group of peripheral vasodilators.* Other NO donors such as sodium nitroprusside and molsidomine are also members of this group.

This list does not exhaust the range of drugs acting as NO donors.

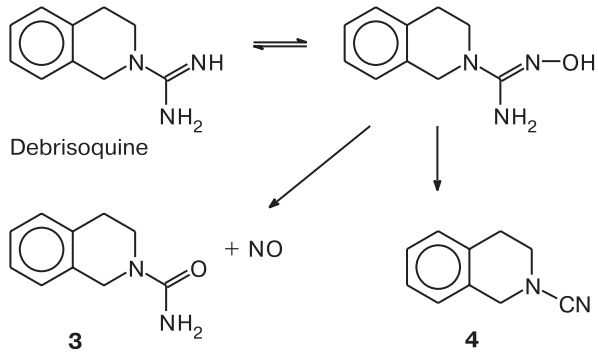
4.1. Guanidine derivatives

It is to this class of compounds that the endogenous nitric oxide donor, L-arginine, belongs. A number of guanidines, nitric oxide donors, have been considered in a previous review.[4] Here, we would like to mention a study devoted to β-aminoethylisothiouronium bromide (**1**), which is readily rearranged at pH 7 into β-mercaptoethylguanidine (**2**).[21] Presumably, oxidation of β-mercaptoethylguanidine to give nitric oxide is followed by its binding to the terminal SH group (Scheme 8).

The nitric oxide formed in the oxidation with potassium ferricyanide was identified based on the polarographically active nitroprusside ion using a procedure specially developed for the detection of NO.[22]

As noted above, NO-synthases represent a special class of monooxygenases related to cytochrome P-450. Oxidation of benzamide oximes and *N*-hydroxyguanidines by cytochrome P-450 is known to give nitric oxide.[16] In addition, it is known that N^{ω}-hydroxy-L-arginine, which is the key intermediate in the endogenous synthesis of NO, is oxidized by cytochrome P-450 to citrulline and NO, this reaction being dependent on the presence of superoxide radical anion.[16,23] Debrisoquine (2-amidino-1,2,3,4-tetrahydroisoquinoline) was chosen[23] as the subject for investigating the ability of "nonphysiological" guanidines to be converted into nitric oxide upon oxidation (Scheme 9). It was found that incubation of this compound with cell structures gave not only the corresponding urea **3**, which is typical of the NO-synthase-mediated oxidation of L-arginine to L-citrulline, but also *N*-cyano derivative **4**. It is of note that *N*-hydroxyguanidines are oxidized to *N*-cyanamides under these conditions, whereas aldoximes are converted into nitriles. The general oxidation pathway of debrisoquine can be represented by Scheme 9.

Scheme 9



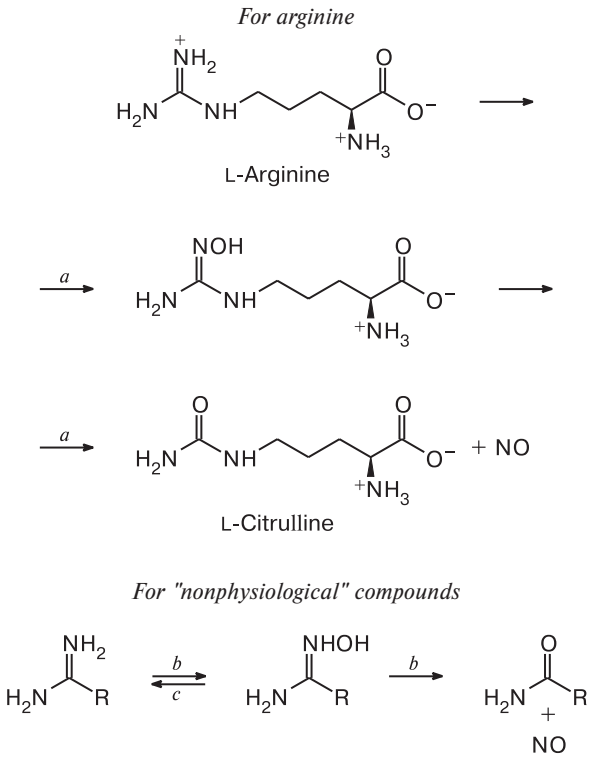
Note that chemical oxidation of *N*-hydroxyguanidines also yields cyanamides.[24]

Exogenous compounds containing amidine fragments can be oxidized by cytochrome P-450 or by a flavin-containing monooxygenase; however, unlike oxidation by NO-synthases, the reverse process, *i.e.*, the reduction of intermediate *N*-hydroxy compounds by reductases, can also take place.[15] The ratio of the rates of these reactions determines the possibility of nitric oxide formation. Therefore, the question of whether a compound can act as an NO donor *in vivo*, cannot be answered *a priori* only on the basis of structural considerations. A comparative oxidation scheme for endogenous and exogenous (nonphysiological) compounds is presented in the study cited[15] (Scheme 10).

The mechanism of cytochrome P-450-catalyzed oxidation is assumed to be similar to that discussed above

* Vasodilators are substances that ensure expansion of blood vessels caused by weakening of the tone of vascular wall muscles.

Scheme 10



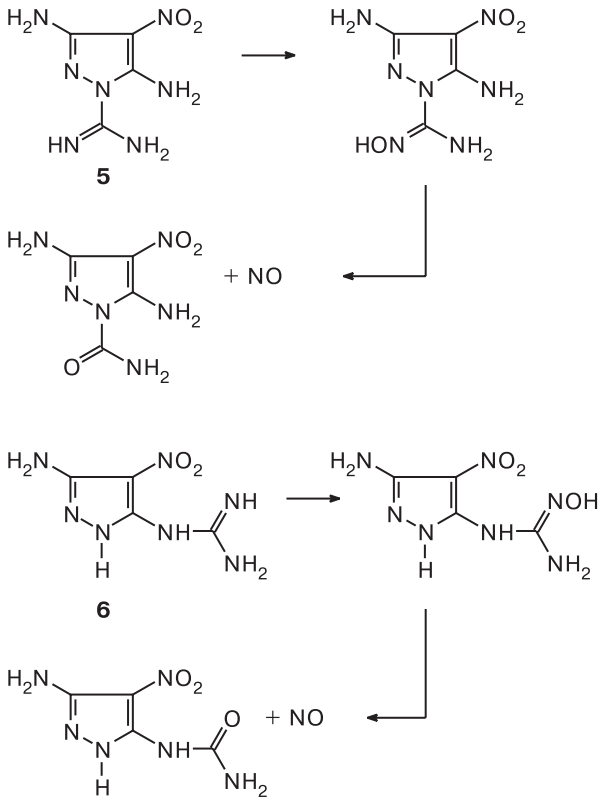
a. NOS, O_2, NADPH; *b*. Cytochrome P-450, O_2, NADPH; *c*. Cytochrome b-reductase, NADH.

for NO-synthase. The difference is that cytochrome P-450 implies the release of superoxide radical anion, which, however, can be destroyed by superoxide dismutase even in the presence of a substrate.[15] An important conclusion drawn from these studies is that exogenous guanidines and related compounds can be considered as promising nitric oxide donors and, hence, compounds of this type are of interest for the search for new drugs.

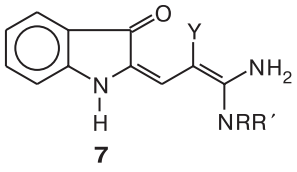
Yet another publication that deserves attention is a recent study dealing with the ability of pyrazole derivatives **5** and **6** containing an amidine or guanidine group as a substituent to undergo oxidative transformations associated with the release of nitric oxide.[25] Oxidation by potassium ferricyanide was carried out in an alkaline medium, and the formation of nitroprusside anion was established by polarography.[22] The release of NO takes place apparently by a usual mechanism, which includes N-hydroxylation as the first step (Scheme 11).

In the previous review,[4] we discussed many investigations dealing with transformations of guanidines into nitric oxide; however, more recently, this approach has no longer been popular; at present, these compounds are studied mainly as NOS inhibitors; this topic is beyond the scope of this review. It is noteworthy that vinylogous guanidine-like compounds **7** found in the series of

Scheme 11



dienediamines of the indoxyl series have exhibited a hypotensive and antithrombotic activity and can act as nitric oxide generators and guanylate cyclase activators. Some of these compounds exhibited high activity as thrombus-formation inhibitors; one of them is currently under extensive preclinical trial.[26,27]

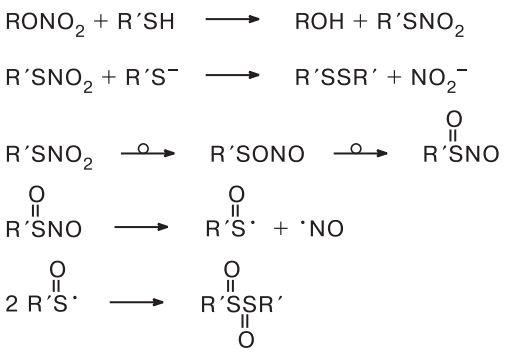


4.2. Nitrates

Nitrates represent yet another class of compounds whose NO-donor properties stipulate their use as drugs; these compounds include nitroglycerin, isorbide dinitrate, isosorbide mononitrate, and pentaerythritol tetranitrate. The pharmacodynamic profiles of nitrates are largely similar; their activity was shown to correlate with lipophilicity.[12] The greater the number of nitro groups, the higher the lipophilicity and the ability to penetrate membranes. The release of nitric oxide starts with the reaction of nitrates with thiols (cysteine, *N*-acetylcysteine), and subsequently NO generators activate soluble guanylate cyclase, the rate of formation of nitric oxide from nitrates being correlated with the sGC activity, *i.e.*, the degree of enzyme activation determines the degree of

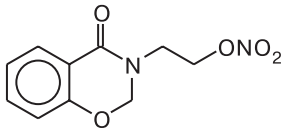
NO release. The pathway of the NO formation from nitrates is shown in Scheme 12. We would like to draw attention to the rearrangement of S-O nitroso compounds[11,12] (see below, in consideration of the biological properties of antimicrobial drugs of the nitrofuran series).

Scheme 12



Before considering new nitric oxide donors belonging to the class of nitrates and then O- and S-nitroso compounds, let us discuss a study pointing to the possibility of formation of new nitro vasodilators in reactions of endogeneous compounds.[28] Peroxinitrite, a very strong oxidant formed in the reaction of NO with superoxide radical anion, is apparently responsible for the cytotoxic action of both reactants. Peroxinitrite $ONOO^-$ affects platelet aggregation, attenuates the action of some vasodilators on the coronary blood circulation, and modifies low-density lipoproteins into forms that contribute to the development of atherosclerosis. However, it was shown that $ONOO^-$ reacts with glutathione to give *S*-nitrosothiol, which regenerates NO. It was suggested that this represents a detoxification pathway for peroxynitrite.[29] The action of peroxynitrite on intact blood vessels induces their relaxation, probably, due to nitrosation of thiols. Such a relaxation is typical of various types of tissues. It has been shown[28] that $ONOO^-$ reacts with D-glucose located in the vascular tissue to give compounds that induce blood vessel relaxation and inhibit platelet aggregation, and these effects can be prevented (as for nitric oxide) by adding oxyhemoglobin. The half-transformation time for the compound formed from peroxynitrite and glucose is much longer than the half-life of NO or $ONOO^-$ (>20, 4, and 2 s, respectively). Thus, another donor of nitric oxide has formed in the abovementioned experiments. Since the experimental conditions ruled out the participation of any thiols, the researchers concluded that peroxynitrite reacted with the hydroxy groups of D-glucose (similar effects were observed in the presence of L-glucose, D-fructose, D,L-glyceraldehyde, and glycerol) to give the corresponding nitrates. This assumption is supported by the fact that the products release a substantial amount of NO in the presence of Cu^+ ions and cysteine, because decomposition of *S*-nitrosothiols is known to be accelerated in the presence of Cu^+ ions. Thus, it appears probable that nitrates are formed in the organism *via* peroxynitrite, although no direct evidence for the occurrence of these processes not only *in vitro* but *in vivo* is presented in the study cited.

Sinitrodil, which is now under preclinical trial, belongs to this type of compound.[30]



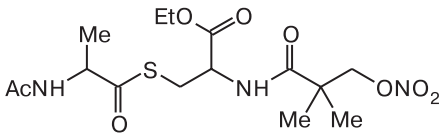
Sinitrodil TTF-296

Typical properties of this drug include apparent antiischemic action, epicardial artery dilatation, and a decrease in the development of tolerance* upon its application. The development of vessel tolerance to nitrates is explained by the fact[31] that the smooth-muscle cells of the vascular wall stop converting nitrates into nitric oxide for reasons not yet entirely known, and, hence, vasodilation does not occur. According to a hypothesis, tolerance may be due to exhaustion of the "cysteine pool", *i.e.*, a decrease in the store of endogenous thiols (cysteine, glutathione) in the cells. No factual data supporting this hypothesis have been found so far. Furthermore, many results contradict it. According to another assumption, the tolerance development is attributed to a decrease in the content or the sensitivity of sGC whose activation is responsible for the formation of cGMP and, ultimately, for vasodilation. It cannot be ruled out that more intense destruction of cGMP by intracellular phosphodiesterase is the probable mechanism for the development of tolerance. According to one more hypothesis, tolerance is due to the release of an enhanced amount of endothelin-1 and superoxide anion, which accelerate NO degradation. Anyway, according to modern views, a disorder of nitric oxide formation (or its accelerated inactivation) is the crucial factor for the tolerance development when nitrates are used as drugs.

A reduced tolerance development has been noted for a series of new drugs; the trend for the selection of vasodilators of this particular type demonstrates the importance of this problem for medicine. A high activity combined with a clear-cut decrease in the tolerance (or even its absence) has been noted for *S*-(*N*-acetyl-D,L-alanyl)-*N*-(2,2-dimethyl-3-nitrooxypropionyl)-L-cysteine ethyl ester (SPM-5185), a compound acting as a nitric oxide

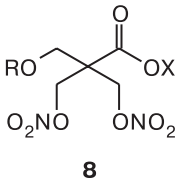
* Tolerance is a decrease in the efficiency of action of pharmaceuticals when they are used repeatedly.

donor with pronounced vasodilatory properties and cardioprotecting effect.[32]

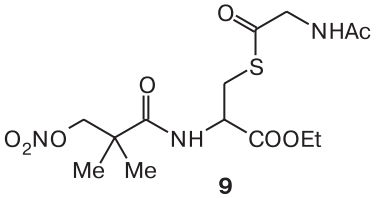


SPM-5185

Among new potential antianginal preparations for treatment of heart failure, mention should be made of a new series of nitrates acting as nitric oxide donors. This is a series of pentaerythritol derivatives **8**,[33] whose pharmacodynamic characteristics are improved compared to those of nitroglycerin or isosorbide mononitrate, and the *N*,*O*-diacyl derivative of cysteine ester **9**,[34] which is an NO donor with a rather long half-life (10 h) that does not change the cardiac beat frequency but improves the contractive functions of heart:



8

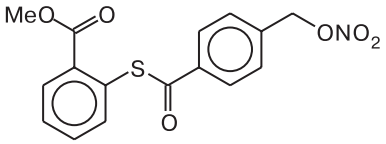


9

8: R = NO_2, X = Na, Me; R = X = Me

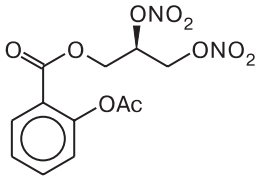
One of pentaerythritol derivatives **8** (R = NO_2, X = Me) exhibits antiatherosclerotic and vasoprotecting effects.[35] This compound releases nitric oxide for a long period of time and does not affect hemodynamics or cause tolerance.

Yet another antianginal drug that is currently under extensive biological testing is mercaptobenzoic acid derivative **10**.[36]

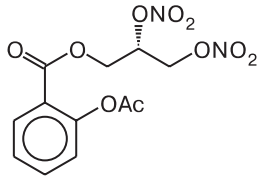


10

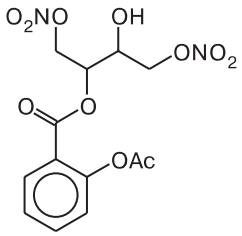
Analgesic and antithrombotic activities were found for a whole series of nitrates, derivatives of acetylsalicylic acid (aspirin) **11—15**.[37]



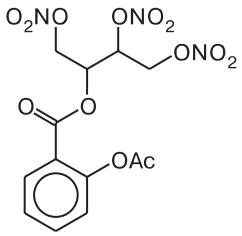
11



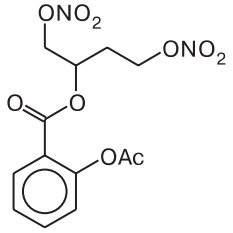
12



13

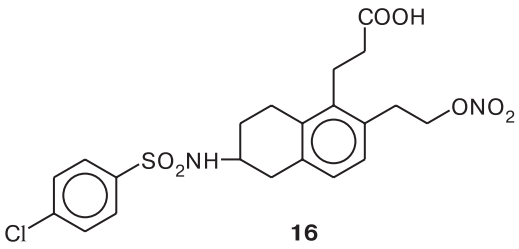


14



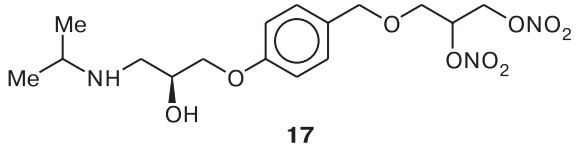
15

A number of *p*-chlorobenzenesulfonamide derivatives intended for treatment of thrombosis and atherosclerosis are under preclinical research. These compounds (for example, **16**) are thromboxane receptor antagonists with NO-donor properties.[38]



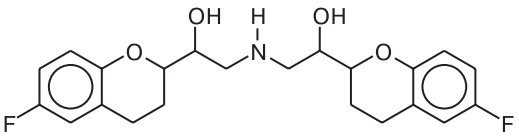
16

One more NO donor described recently induces long-term vasorelaxation with slow release of NO, having an activity of the order of nitroglycerin activity. In addition, this is a more efficient β-adrenergic blocker than some known drugs such as metoprolol and atenolol. The main targets for the action of compound **17** include ischemic heart diseases, hypertension, and atherosclerosis.[39]



17

A vasodilatory effect is inherent in one more compound whose structure combines fragments typical of β-adrenergic blockers and NO donors.[40] The interest in this type of compound is stimulated, apart from the obvious theoretical aspects, by the fact that a new β_1-adrenoreceptor blocker, nebivolol, introduced recently into clinical practice, stimulates the synthesis of endogenous nitric oxide, which provides a beneficial effect in the therapy of hypertension.

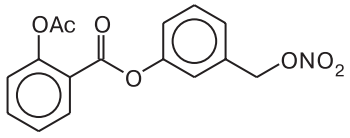


Nebivolol

Compound **18** has been synthesized as an analog of propranolol (β-adrenergic blocker).[40] Presumably, the mechanism of action of this compound includes its reaction with endogenous cysteine to give *S*-nitrocysteine. This product rearranges into *S*-nitroso *S*-oxide, which is transformed into cysteine *S*-oxide radical (which probably dimerizes) and nitric oxide (Scheme 13).

However, pharmacological study of this "β-adrenergic blocker—NO donor" hybrid did not show statistically valid differences between its action and the action of propranolol as regards the blood pressure reduction.

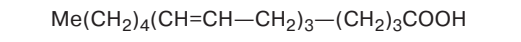
Clinical trial is now carried out for aspirin derivative **19**.



19

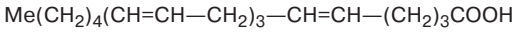
Nitric oxide is known to be involved in the gastrointestinal tract protection. In addition, various (both positive and negative) effects of acetylsalicylic acid are known[41] to be related to its influence on prostaglandin biosynthesis, metabolism, and release. These factors are responsible for the antiaggregatory activity of this drug, *i.e.*, the ability to inhibit platelet aggregation, which finds extensive use for the prevention of various thromboses, *viz.*, postsurgical and stroke-caused thromboses, thrombophlebitis, and thrombus formation caused by the ischemic cardiac disease or cardiac infarction. However, the retardation of prostaglandin synthesis causes a number of complications, most important of them being an increase in the gastric acidity, the disturbance of mucous tunic blood supply, dyspeptic disorders, gastric hematocelia, and provocations of erosive and ulcerous processes (ulcerogenic action). Since such a risk exists not only for acetylsalicylic acid but also for other nonsteroidal antiinflammatory drugs, it is pertinent to shed light on some aspects related to biosynthesis and the role of prostaglandins in the living organism.

Prostaglandins are second messengers that transform the action of hormones or neuromediators into the corresponding physiological response. Usually, their operation is strong but short-term because they are quickly destroyed by specific dehydrogenases. γ-Linolenic acid gets into the organism with food and is then converted into arachidonic acid, which is accumulated in cell membrane phospholipids and is released under the action of phospholipase A_2.



$Me(CH_2)_4(CH{=}CH{-}CH_2)_3{-}(CH_2)_3COOH$

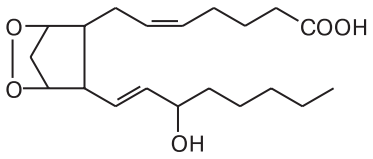
γ-Linolenic acid



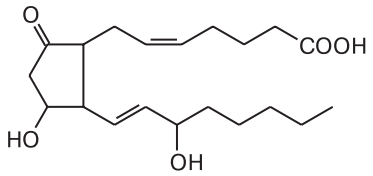
$Me(CH_2)_4(CH{=}CH{-}CH_2)_3{-}CH{=}CH{-}(CH_2)_3COOH$

Arachidonic acid

The metabolism of arachidonic acid induced by cyclooxygenase results in endoperoxide PGG_2, which is transformed with participation of prostaglandin synthase to give prostaglandins E and F (prostaglandins E have an oxo group in position 9 and a hydroxy group in position 11, while in prostaglandins F, hydroxy groups are present both in positions 9 and 11).

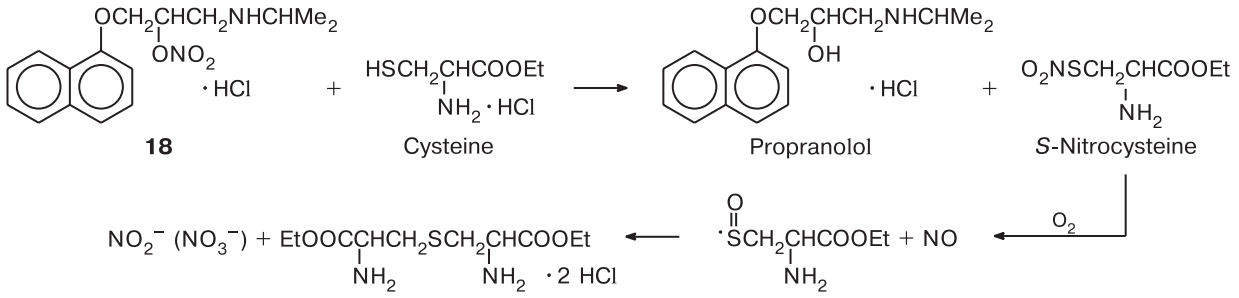


Endoperoxide PGG_2

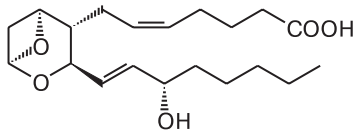


Prostaglandin E_2 (dinoprostone)

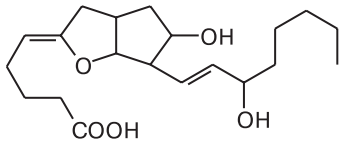
Scheme 13



Under the action of thromboxane synthase, endoperoxide is converted into thromboxane A_2, responsible for thrombus formation in vessels. Simultaneously, the third enzyme, prostacyclin synthase transforms the same peroxide into epoprostenol (prostacyclin), which prevents thrombus formation; this creates the balance needed for life sustenance.



Thromboxane A_2



Prostacyclin

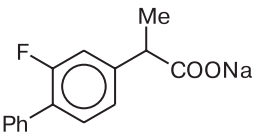
Prostaglandins play an important role in physiological and pathological processes in tissues. Therefore, retardation of prostaglandin synthesis in the stomach wall, where they control its protective properties by maintaining blood supply and decreasing hydrochloric acid secretion, provokes the ulcerogenic effect mentioned above.

The irreversible inhibition of prostaglandin synthase by nonsteroidal antiinflammatory drugs brings about side effects associated with stomach disorder. However, a reduced prostaglandin synthesis results in normalization of rheumatism-affected tissues.

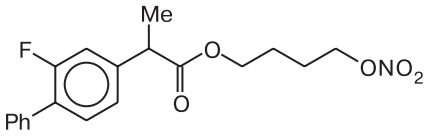
A fairly dangerous complication caused by the use of nonsteroidal antiinflammatory drugs is bronchospasm. The blockade of prostaglandin synthesis can direct the arachidonic acid metabolism toward leukotrienes (tissue edema, smooth muscle spasm).

Since nitric oxide exerts an effect similar to that of prostaglandins, the idea of incorporating an NO-donor group into the aspirin molecule attracts increasing attention. Compound **13**, whose structure contains both an acetylsalicylic acid fragment and a nitrate-containing substituent, actually proved to be an efficient antiinflammatory and antithrombotic drug and an active gastroprotector.[42]

A similar goal was pursued in the synthesis of a new drug based on flurbiprofen possessing an antiinflammatory activity. The compound prepared, HCT 1026 (phase II clinical trial) efficiently protects the mucous membrane of the stomach and decreases inflammatory processes in brain. Due to drug´s influence on the brain, the researchers arrived at the idea that a strategy of using intiinflammatory drugs in the therapy of mental disorders such as Alzheimer´s disease could be developed on the basis of this drug.[43]

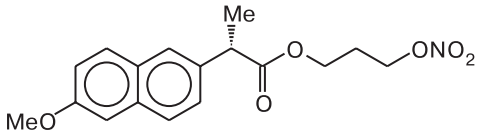


Flurbiprofen

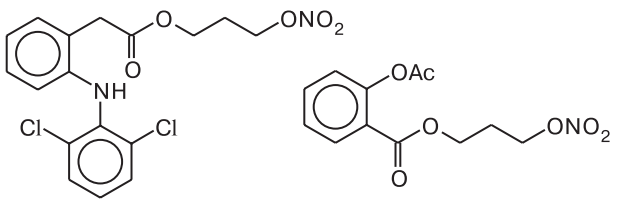


HCT-1026

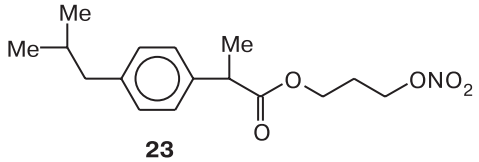
The structures of some other antiinflammatory drugs containing an NO-donor fragment are shown below (**20**—**24**):



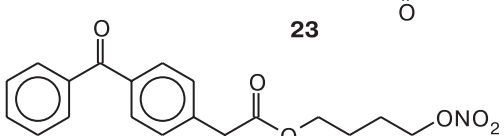
20



21 **22**



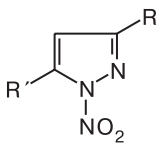
23



24

4.3. *N*-Nitro derivatives

The biological action of *N*-nitro derivatives largely resembles the action of nitrates. *N*-Nitropyrazoles **25** have been studied in detail.[44]



25

In experiments on isolated rat aorta, these compounds exert a dose-dependent decrease in the noradrenaline-caused smooth muscle tension. They are not inferior to isosorbide dinitrate and are superior to isosorbide mononitrate in spasmolytic activity. All the *N*-nitropyrazoles studied, in a dose of 50 mg kg^{-1}, decrease the blood pressure in narcotized rats with

nephrovascular hypertension by 25—30 Torr and differ only in the pattern of the effect development. Chemical reduction of cysteine or potassium ferrocyanide gives nitric oxide (identified as the polarographically active nitroprusside anion) (Scheme 14).

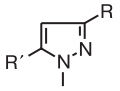
Scheme 14

$$PzNO_2 + 2\ R''SH \longrightarrow PzH + R''SSR'' + HNO_2$$

$$R''SH + HNO_2 \longrightarrow R''SNO + H_2O$$

$$2\ R''SNO \longrightarrow R''SSR'' + NO$$

Pz =



Reduction with ferrocyanide follows the equation

$$PzNO_2 + 3\ K_4[Fe(CN)_6] + H_2O \longrightarrow$$

$$\longrightarrow 2\ K_3[Fe(CN)_6] + K_2[Fe(CN)_5NO] +$$

$$+ KCN + 3\ KOH + PzH$$

All the *N*-nitropyrazoles studied activate soluble guanylate cyclase. Further investigation of these compounds showed that they exert a beneficial effect on the eye blood flow and the retina function; *N*-nitropyrazoles restore these functions after an ischemic stroke.[45,46]

4.4. Organic nitrites

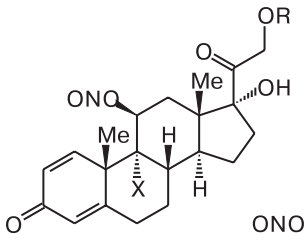
Organic nitrites are hydrolytically less stable than nitrates and react smoothly with thiols to release nitric oxide (Scheme 15).[12]

Scheme 15

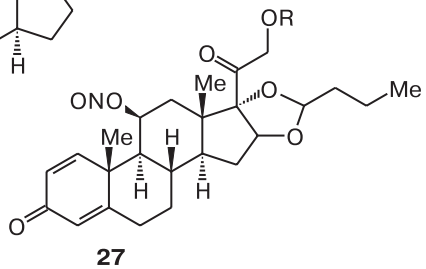
$$RONO + R'SH \longrightarrow R'SNO + ROH$$

$$2\ R'SNO \longrightarrow R'SSR' + 2\ NO$$

In recent years, a number of compounds (**26**, **27**) functioning as nitric oxide donors and exhibiting interesting and promising biological properties have been found among *O*-nitroso derivatives. This type of compound includes combined-action drugs that possess both the antiinflammatory activity of glucocorticoids and the vasodilatory effect of nitric oxide donors.[47]

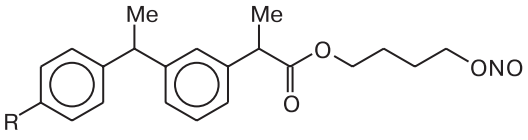


26



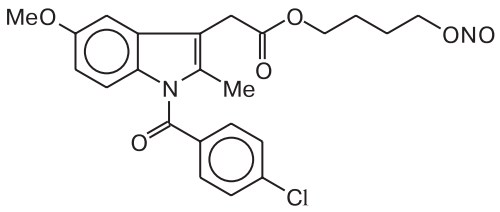
27

A number of nonsteroidal antiinflammatory drugs (NSAID) (**28**) have been synthesized on the basis of compounds containing an aryl- or hetarylacetic acid fragment, which is standard for NSAID (in particular, for the well-known drug indometacin), and a residue containing an *O*-nitroso group.[48] When considering the biological activities of these compounds, their low damaging effect on the gastrointestinal tract is specially emphasized.



28

R = H, Me_2CHCH_2



Indometacin derivative

4.5. *S*-Nitrosothiols

S-Nitroso compounds form one of the vastest and most extensively studied groups of nitric oxide donors.

The functions of *S*-nitrosothiols in the organism are fairly diverse. Presumably, they play the role of the endothelium-derived relaxing factor (EDRF).* However, there is also a different view on the forms of nitric oxide stabilization in biological systems; apart from *S*-nitrosothiols, these include iron dinitrosyl complexes (IDNC). Interconversions of these compounds determine the NO transport in cells and tissues.[5] The neuroprotective properties of *S*-nitrosothiols (and related compounds) are attributable to their ability to undergo *trans*-nitrosation with the thiols incorporated in NMDA-receptors,** whereas the neurodestructive effects of NO are related to

* The endothelial relaxing factor is a substance formed in the vessel endothelium and capable of inducing vasodilation. Now it has become obvious that this is nitric oxide or, more precisely, the forms of its deposition in the body.

** NMDA receptor is a type of receptor that enables realization of the effects of the main exciting amino acid, glutamic acid. The agonist of this receptor is *N*-methyl-D-aspartic acid (NMDA).

the interaction of *S*-nitrosothiols with superoxide to give peroxynitrite. Important data on the biological action of *S*-nitrosothiols have been reported.[49] It was found that biological effects of many compounds of this type depend little on the rate of NO release. It was shown[50] that the stimulation of sGC is governed by the presence of NO^+. Thus, the effects of *S*-nitrosothiols are beyond the sGC activation alone, and decomposition of these compounds in the organism gives not only the nitric oxide radical and the corresponding cation but also the nitroxide anion NO^-; in other words, the S—N bond undergoes not only homolytic but also heterolytic cleavage.

$$RS^- + NO^+ \longleftarrow RSNO \longrightarrow RS^{\cdot} + NO^{\cdot}$$

It should be noted that the nitrosonium cation is said to play a conflicting role in brain, *i.e.*, it is able to act not only as a neuroprotector but also as a neurotoxic agent.[51] The NO^+ cation is too reactive to exist in an aqueous medium where it is readily converted into HNO_2. However, this cation is assumed to be an intermediate in the *trans*-nitrosation[51] (Scheme 16).

Scheme 16

$$NO^+ + H_2O \longrightarrow HNO_2 + H^+$$

$$RSNO + R'S^- \longrightarrow RS^- + R'SNO$$

The biological role of the nitroxide ion, which is easily formed from nitric oxide under the action of superoxide dismutase, is unknown.

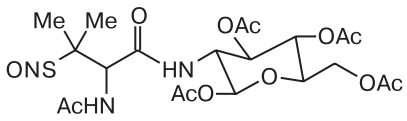
When discussing the possible use of *S*-nitrosothiols as new drugs, one should borne in mind that the homolytic cleavage of these compounds (under the action of heat, light, change in the pH, or oxygen) (Scheme 17) gives not only disulfides and NO but also thiyl radicals, which can, in principle, undergo other reactions, apart from dimerization, including undesirable reactions.

Scheme 17

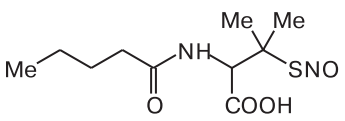
$$RSNO \longrightarrow RS^{\cdot} + NO$$

$$2\,RS^{\cdot} \longrightarrow RSSR$$

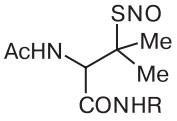
Yet another problem is the possibility that nitrosothiols would perform *trans*-nitrosation of the free HS groups of proteins, which can result in irreversible modifications to give mixed disulfides and in the loss of enzymatic activity.[12] Thus, there exist a number of serious obstacles hampering the use of *S*-nitrosothiols in medicine. The most rational way to resolve these problems is to find compounds that would selectively affect particular organs and tissues. Therefore, researchers are faced with the challenge of preparing compounds that would possess clearly more favorable properties than the known nitric oxide donors of the given series. The search for these compounds is carried out fairly intensively. Recently, the synthesis and study of some biological properties of new *S*-nitrosothiols including penicillamine derivatives (RIG 200, D-SNVP) and *S*-nitroso dipeptides **29** have been performed.[52—58]



RIG 200



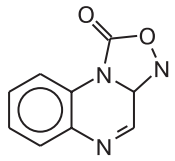
D-SNVP



29

R = Gly, Ala, Val, Leu, Phe, Ile, Met, Pro, Asn, Gln residues

RIG 200 and D-SNVP are much more stable than one of the first NO donors of this series, *N*-acetyl-*S*-nitrosopenicillamine (SNAP) in aqueous solutions (the half-life of SNAP is ~40 min and those of RIG 200 and D-SNVP, are more than 220 min). These donors are less sensitive to traces of Cu^+; they have a pronounced vasodilatory action and do not cause tolerance. It is significant that the activity of these compounds does not diminish when they act on blood vessels that are tolerant to the action of nitrates. A special study demonstrated the efficiency of RIG 200 for the therapy of damaged blood vessels.[54] *S*-Nitroso dipeptide derivatives **29** are more stable against decomposition in the presence of Cu^+, because the constants of formation of copper complexes are lower than those for SNAP and *S*-nitrosoglutathione (GSNO). Therefore, the vasodilatory effect of these compounds is higher than those of the known NO donors of this series (SNAP, GSNO). However, in the presence of hemoglobin, the effects of the above-considered nitric oxide generators are leveled, and further search for compounds of this type acceptable for the practical use is needed.[57] While returning to RIG 200 and D-SNVP, one should note that, unlike GSNO, they are not subject to the influence of ferrohemoglobin. This has been attributed[58] to the fact that the action of these drugs is concentrated at the sites where the access of ferrohemoglobin is limited. Meanwhile, vasodilation induced by any of the *S*-nitroso compounds considered is completely removed by an sGC inhibitor, 3a,10-dihydro-1*H*-[1,2,4]-

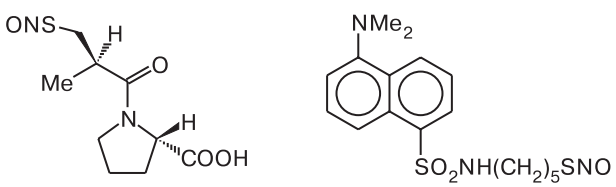


ODQ

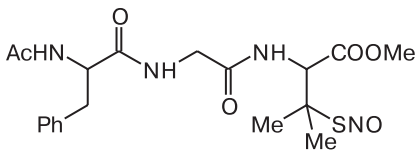
oxadiazole[4,3-*a*]quinoxalin-1-one (ODQ),[59] *i.e.*, the effect of these compounds is directly related to their ability to activate sGC.

It is often difficult to interpret unambiguously the effects of biologically active compounds. Recently, it has been found[60] that ODQ is not a specific inhibitor of only sGC but it also interferes with other heme-dependent processes. It is demonstrated in the study cited that, apart from the action on sGC, ODQ affects vasorelaxation by other mechanisms. Therefore, the partial or complete inhibition of the biological response by ODQ can be misinterpreted as necessary inclusion of cGMP in the formation of this response. It is the absence of a reaction to the introduction of ODQ that indicates that neither cGMP nor the ODQ-sensitive mechanism with participation of cytochrome P-450 are involved in the effects of the drug under study. The problems related to the mechanisms of action of NO donors that require preliminary metabolic activation, in particular redox-activation, should be approached with special caution. For compounds of the *S*-nitrosothiol type, which do not require this activation, ODQ is an important indicator which shows the degree of participation of sGC in vasodilatory effects.

S-Nitrosothiols are able to react with the HS groups of protein molecules; this exerts an ambiguous influence on the protein functions. Cysteine proteases, a large group of plant, animal, and bacterial enzymes, are known to play an important role in various biological processes. Many disease states such as muscular dystrophy, various inflammations, or rheumatoid arthritis can be related to an increased proteolytic activity of cysteine proteases. There exists an actual demand in the development of selective inhibitors of these enzymes. Papain is one of the best studied enzymes of this class. The active site of this enzyme contains Cys^{25} and His^{159}, and it is assumed that the catalytically active thiolate—imidazolium ion pair can be sensitive to NO donors. It was shown that nitric oxide generators such as *N*-nitrosoanilines,[61,62] sodium nitroprusside, and (3*E*)-4-ethyl-2-hydroxyimino-5-nitrohex-3-enamide (FK-409)[63] (see below) are effective papain inhibitors, inactivation of the enzyme being due to the formation of the stable S—NO bond. The inhibitory action of *S*-nitrosothiols such as SNAP, GSNO, and RIG 200 (see above), *S*-nitrosocaptopril, *N*-(*N*-acetylphenylalanylglycyl)-*S*-nitrosopenicillamine methyl ether, and *N*-(5-dimethylaminonaphthalene-1-sulfonyl)-*S*-nitroso-5-aminopentane-1-thiol has been studied.[64] *S*-Nitrosocaptopril (like compound **22**) is a drug of combined action, manifesting both the nitric oxide donor properties and an antihypertensive activity typical of angiotensin-converting enzyme inhibitors such as a well-known captopril (capoten).[41]

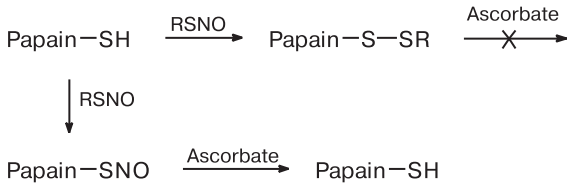


S-Nitrosocaptopril



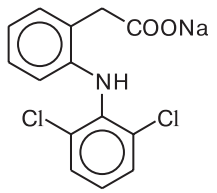
S-Nitrosothiols were shown to be effective papain inhibitors (Scheme 18).[64]

Scheme 18

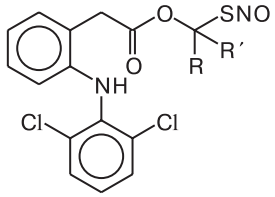


Thus, one more effect caused by NO donors, namely, inactivation of cysteine proteases, opens up a new line in the rational search for effective drugs.

Derivatives of diclofenac sodium **30** containing an *S*-nitrosothio fragment have been prepared.[65]



Diclofenac sodium

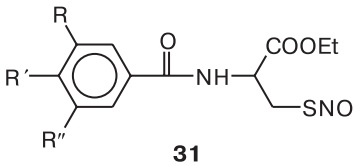


30

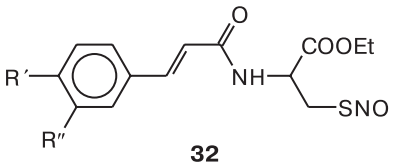
A biological assay of these nitric oxide donors has shown that they retain high antiinflammatory and analgesic activities typical of diclofenac but have a much less pronounced ulcerogenic action. Presumably, the release of NO from these compounds prevents the activation of gastric cysteine protease, and this gives rise to the aforementioned beneficial effects. The ability of nitric oxide to prevent gastrointestinal disturbances and to accelerate the cure of stomach and duodenal ulcers forms one of the most promising lines of research in the use of NO donors.[66]

An abnormally high content of nitric oxide may induce deleterious effects, which are often related to the formation of peroxynitrite upon the reaction of NO with superoxide. When introducing exogenous NO donors in the organism, one should also keep in mind that natural carriers of nitric oxide such as S-nitrosoalbumin, SNAP,

and GSNO are always present in the cells.[67] In order to avoid excess nitric oxide, it appears expedient that NO-donor molecules incorporated "internal carriers" able to capture the "surplus" nitric oxide and to exclude the presence of superoxide and peroxynitrite in the system. In view of this reasoning, a number of new *S*-nitrosothiols (**31**, **32**) whose molecules contain phenolic groups typical of antioxidants have been synthesized.[68]



31



32

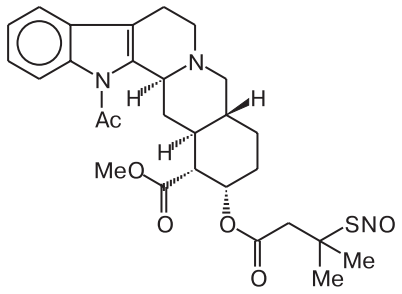
31: R = H, R´ = OH, R″ = OMe; R = H, R´ = OMe, R″ = OH; R = R″ = Bu^t, R´ = OH

32: R´ = OH, R″ = OMe; R´ = OMe, R″ = OH

It was shown[68] that disulfides **33**, formed upon cleavage of these compounds with nitric oxide release, are superoxide radical anion traps; the reaction between these species yields radicals of the type **34** (Scheme 19).

Only detailed pharmacological analysis of the resulting compounds can show to what extent this idea is fruitful.

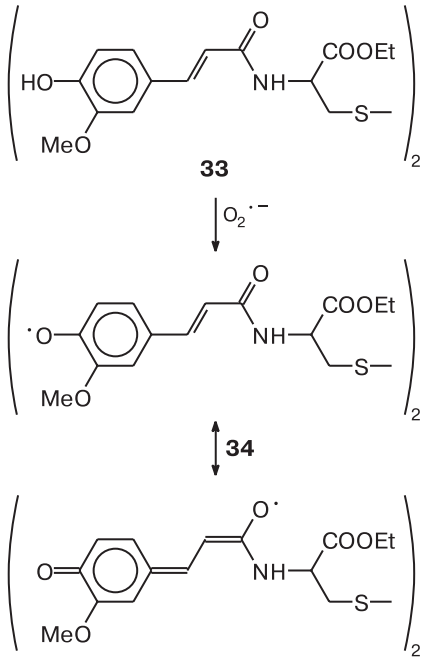
New promising nitric oxide donors, *S*-nitroso derivatives, functioning as α_2-adrenergic receptor antagonists* and meant for the treatment of impotence have been prepared.[69] Yohimbine derivative **35** is a representative of this group (α_2 : α_1-selectivity = 10 : 1).



35

Fairly interesting results were obtained in a study of a number of polypeptide compounds containing an SNO group.[70] These polypeptides exhibit a strong vasodilatory action; moreover, which is very important, the vasorelaxation in some organs (for example, in the rat aorta) is associated with an increase in the cGMP level, while in other organs (trachea), it is related to an increase in the cAMP concentration.

Scheme 19



The above-mentioned RIG 200, characterized by slow release of nitric oxide, is under clininal investigation.[71]

A study of *S*-nitroso-*N*-acetylpenicillamine (SNAP)[72] showed the ability of nitric oxide donors to increase the level of dopamine, which is the noradrenaline precursor in the biosynthesis, in organs and tissues. The involvement of NO in the regulation of metabolism of catecholamines was established previously in a study of their reactivity toward nitric oxide, nitrite, and peroxynitrite.[73] The processes involved are presented in Scheme 20.

It was found that peroxynitrite not only acts as a nitrating agent but also oxidizes catecholamines; this is considered to be responsible for neurotoxicity of peroxynitrite at Parkinson´s disease or at senescence.

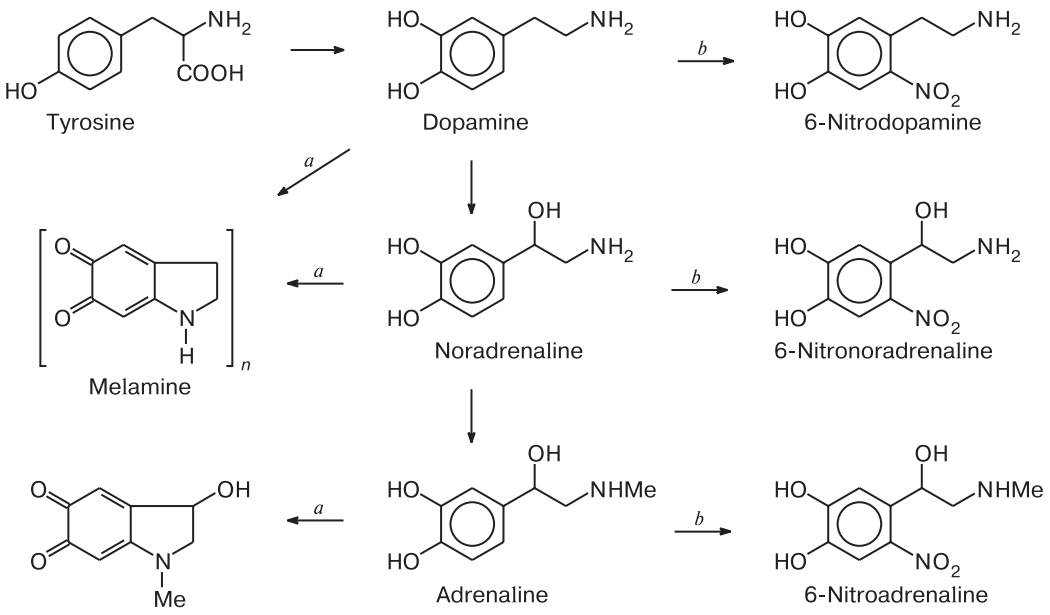
In the organism, *S*-nitrosothiols are transformed into nitric oxide in different ways. These include thermal or photochemical decomposition and decomposition induced by metal cations, first of all, by the monovalent copper cation.[74,75]

$$RSH + HNO_2 \xrightarrow[-H_2O]{} RSNO \xrightarrow{h\nu \text{ or } \Delta} RSSR + 2\,NO$$

$$2\,RS^- + 2\,Cu^{2+} \longrightarrow RSSR + 2\,Cu^+ \xrightarrow{2\,RSNO} 2\,Cu^{2+} + 2\,RS^- + 2\,NO$$

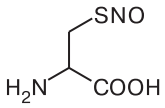
* The α_2-adrenergic receptor is a subtype of adrenergic receptors (noradrenaline is the mediator) whose stimulation induces a short-term pressor (vasoconstrictive) effect and a long-term hypotensive (vasorelaxing) effect.

Scheme 20

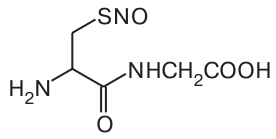


a. $ONOO^-$, pH 7.4; *b*. NO, O_2, pH 7.4.

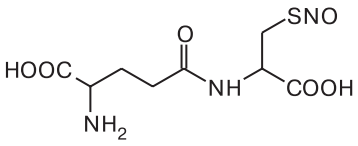
The dependence of the copper-catalyzed decomposition of *S*-nitrosothiols on the structure of substrates containing cysteine or glutathione fragments has been studied.[74] The type of structure was found to play an important role in the stabilization (or destabilizastion) of these compounds.



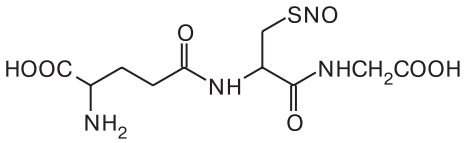
S-Nitrosocysteine (**36**)



S-Nitrosocysteinylglycine (**37**)

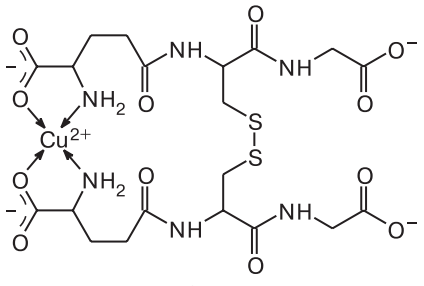


S-Nitrosoglutamylcysteine (**38**)



S-Nitrosoglutathione (**39**)

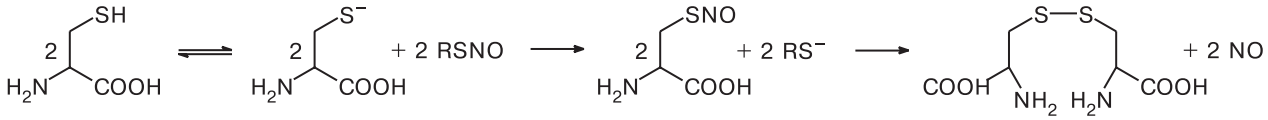
It was found that cysteine derivatives **36** and **37** present in high (millimole) concentrations rapidly decompose in the presence of Cu^{2+} ions, while compounds **38** and **39**, containing the glutamic acid residue, decompose very slowly under these conditions. When present in low (micromole) concentrations, all nitrosothiols decompose equally rapidly. This was attributed to the fact that the glutamate residues are responsible for the complexation with Cu^{2+}; at high concentrations, Cu^{2+} ions are completely consumed and decomposition of peptides **38** and **39** ceases. At low concentrations, the complexation, including the formation of complexes with disulfides arising in the reactions, is less significant. In the case of disulfide derived from glutamate **39**, structure **40** was proposed for the resulting complex.



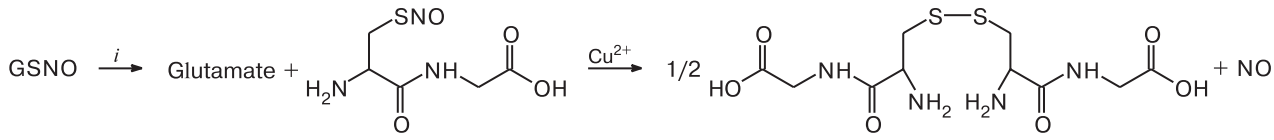
40

S-Nitroso compounds can also undergo *trans*-nitrosation. The transfer of the nitroso group from relatively

Scheme 21



Scheme 22



i. Glutamyltranspeptidase

stable *S*-nitrosothiol to, for example, cysteine gives rise to unstable *S*-nitrosocysteine and further, in the presence of Cu^+, to nitric oxide. Thus, accelerated formation of NO takes place in those cases where the initial (in particular, endogenous) *S*-nitrosothiols are transformed too slowly into nitric oxide (Scheme 21).

Endogenous *S*-nitroso peptides are cleaved under the action of either enzymes (Scheme 22) or ascorbic acid.

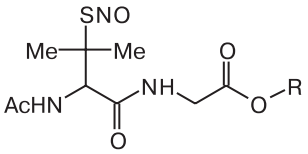
The latter can act according to two mechanisms: at low concentrations, it reduces Cu^{2+} to Cu^+ thus catalyzing degradation of SNO derivatives, while at high concentrations, it acts as a nucleophile by attacking the *S*-nitroso group and increasing the release of nitric oxide.[11]

Now it is clear that *S*-nitroso derivatives of amino acids, peptides, and proteins are products of nitric oxide metabolism in the organism. These endogenous compounds (whose formation might be a way of deposition of NO in the organism) include the *S*-nitroso derivative of serum albumin; a high level of *S*-nitrosoglutathione was found in the human bronchial liquid. It is not exactly known yet how endogenous *S*-nitroso compounds are formed and released. However, it is quite probable that a number of pathologies are due to the deficiency of NO in the organism, and it is necessary to continue the search for new exogenous sources of nitric oxide.

The most important objectives of the synthesis of "improved" NO donors of this series include (1) increasing the stability of *S*-nitroso compounds during storage (without the change in the ability to produce NO *in vivo*), (2) increasing their lipophilicity in order to facilitate their penetration through cell membranes, (3) ensuring high purity and simplicity of the synthesis, and (4) minimization of optical isomerism.[76]

The synthesis of a number of new nitric oxide donors, *S*-nitroso dipeptides **41**, was reported.[76]

The chemical stability of these compounds in the presence of Cu^{2+} is higher than the stability of SNAP and GSNO as model compounds. Nevertheless, their vasodilatory effect is much more pronounced than the effect of SNAP. Meanwhile, the antiaggregatory activities of the

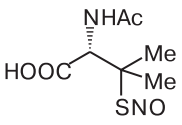


41

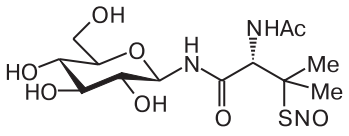
R = Me, Et, Pr^n, Pr^i, Bu^n, Bu^t

new and the model compounds were virtually the same. This means that not only the decomposition rate but also other factors can play an important role in the manifestation of the biological action, and these factors are not identical for blood vessels and platelets. The results obtained reflect the differences between the abilities of these substances to penetrate cell membranes and to participate in the intracellular mechanisms of nitric oxide release in various organs and tissues. In other words, we are dealing with tissue specificity, which is a very important parameter in the clinical investigation of new compounds.

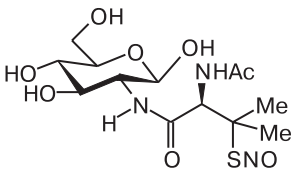
Since the presence of a carbohydrate fragment normally ensures better solubility, enhanced transport through cell membranes, and effective interaction with receptor systems, a series of glyco *S*-nitrosothiols **42—46** have been synthesized by amidation of SNAP with glycosylamines and amino sugars.



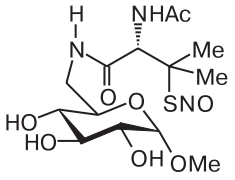
SNAP



42

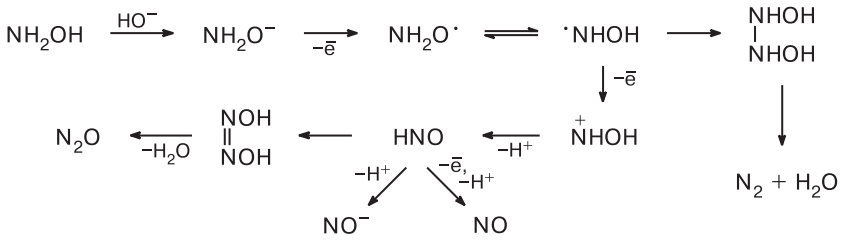


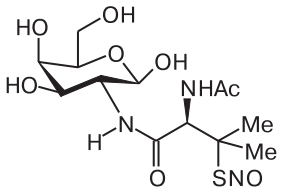
43



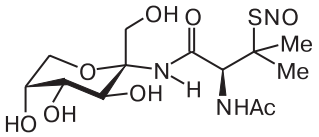
44

Scheme 23



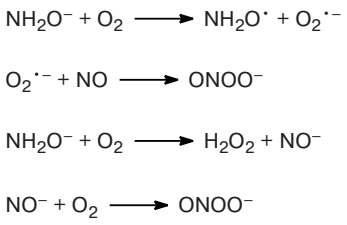


45

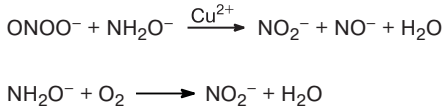


46

Scheme 24



Scheme 25



Preliminary trials of the synthesized compounds showed that glucosylamine and 2-amino-2-deoxyglucose derivatives generate nitric oxide for much longer periods than SNAP itself.

4.6. Hydroxylamine derivatives

The diversity of both beneficial and adverse effects of nitric oxide associated with interconversions of its oxidized and reduced form provided grounds for proposing the concept of nitric oxide cycle in the organism.[77] Reasoning from the formula of hydroxylamine, it can be easily suggested that it could be converted into nitric oxide under appropriate conditions. This aspect has been studied in detail.[78] It was found that oxidation of NH_2OH yields nitrogen, nitrogen oxides N_2O and NO (*via* HNO), nitrite, and nitrate, their ratio being determined by the nature and concentration of the oxidant, the medium pH, the temperature, and the nature of the solvent. The hypothetical sequence of oxidation of hydroxylamine with potassium ferricyanide (with a polarographic detection of NO as nitroprusside anion) is shown in Scheme 23.

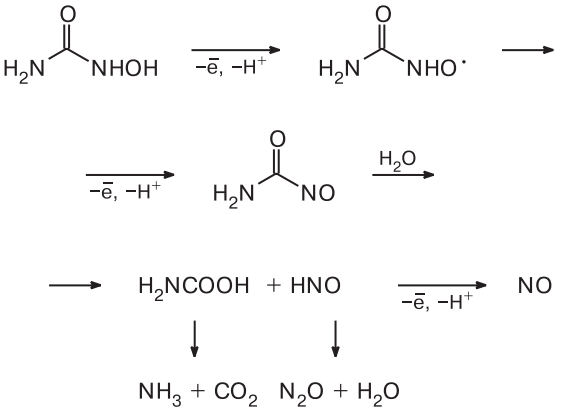
If oxygen is used as the oxidant, hydroxylamine is partially converted into peroxynitrite (Scheme 24).

Transition metal cations (first of all, Cu^{2+}) catalyze the following processes (Scheme 25).

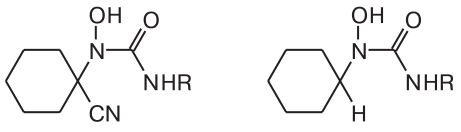
Constant bubbling of oxygen into an aqueous solution of hydroxylamine (10^{-2} mol L^{-1}) at pH 12—13 at 20 °C results in its oxidation over a period of several hours. Peroxynitrite arises as an intermediate, but by the end of the process, it disappears, and NO_3^- is produced as the major product, its yield being ~75%. The intermediate formation of peroxynitrite can be judged based on the nitration products formed. For example, if the medium contains tyrosine, it is easily converted into 6-nitro derivative.[79] Peroxynitrite was identified[78] by detecting *o*- and *p*-nitrophenols, which result from the reaction of this species with phenol. Since hydroxylamine can be oxidized to give NO, the antithrombotic and antihypertensive actions of a number of hydroxylamine and *N*-phenylhydroxyamine derivatives have been studied.[80] Hydroxylamine itself is a hydrophilic compound, poorly absorbed from the gastrointestinal tract and rapidly reacting with endogenous carbonyl compounds; among other factors, the latter accounts for its substantial toxic action. A large group of *N*- and *O*-substituted lipophilic hydroxylamines were synthesized.[80] It was shown that *N*-ethoxycarbonyl-*N*-phenylhydroxylamine having the highest antithrombotic activity does not exhibit vasodilatory activity. Functionally substituted hydroxylamines are of interest as nitric oxide donors. The research logic resulted in testing *N*-hydroxyurea (HU), which has been used for treatment of some types of cancer, as an NO donor.[81] It is considered at present that the mechanism of action of HU is related to its ability to inhibit (or

block) the DNA synthesis by inhibiting ribonucleotide reductase, which is responsible for the reduction of the 2´-OH group in ribonucleotides to deoxyribonucleotides. A study[82] of HU oxidation by potassium ferricyanide with polarographic detection of NO (based on the formation of the nitroprusside ion) has shown that HU is an NO donor. The nitric oxide is released according to the following hypothetical pattern (Scheme 26).

Scheme 26



It was suggested that HU reacts with the hemoprotein heme with *in vitro* generation of NO.[83] The formation of nitric oxide upon introduction of hydroxyurea was also established *in vivo*; it is catalyzed by heme-containing proteins such as P-450.[84] *N*-Hydroxyurea does not exert antithrombotic action; an attempt was undertaken[85] to design a molecule containing not only a hydroxyureide fragment, but also electron-withdrawing and membranotropic substituents. The major idea was that these substituents would increase absorption of compounds from the gastrointestinal tract, ensure the affinity to lipophilic targets such as liver and platelets, and enhance release of nitric oxide. In this study,[85] a group of compounds of type **47** were prepared, and it was shown that manifestation of the desired biological action requires the presence of a geminal cyano group and the hydroxyureide fragment.

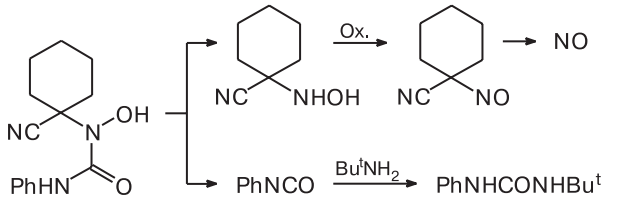


47

Active Inactive

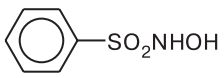
These compounds inhibit thrombus formation (the most active compounds are those in which R is hexyl or phenyl), which is attributed to their ability to generate NO. The amount of nitric oxide formed spontaneously was shown to be small but the addition of an Fe^{3+}—porphyrin complex and an oxidant markedly enhances NO formation (more substantially than in the case of SIN-1). The authors proposed a route to nitric oxide (Scheme 27).

Scheme 27

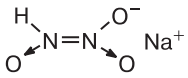


The formation of phenyl isocyanate was proven by the preparation of *N*-*tert*-butyl-*N*´-phenylurea.

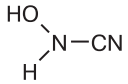
It can be seen from the studies discussed above that nitric oxide might be released through the intermediate formation of HNO, which can act as an NO donor itself. In this connection, one should dwell on compounds such as Piloty´s acid, Angeli´s salt, and *N*-hydroxycyanamide. The incorporation, into systems involving nitric oxide, of its single-electron redox forms, *viz.*, the oxidized form, nitrosonium cation (NO^+), and the reduced form, nitroxyl anion (NO^-), or its conjugate acid, HNO, partially accounts for the reactivity of NO with respect to various biological targets and its broad biological action. The biological effects of Piloty´s acid, Angeli´s salt, and *N*-hydroxycyanamide mimic those of NO.



Piloty´s acid



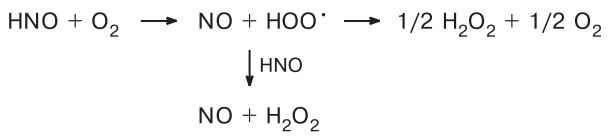
Angeli´s salt



N-Hydroxycyanamide

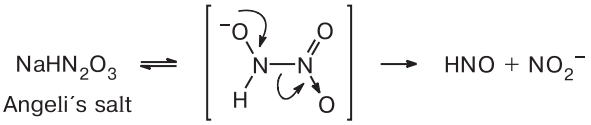
It was established that these compounds release HNO and cause relaxation of smooth-muscle vessels, the vasodilatory action being accompanied by accumulation of cGMP.[86] Presumably, many effects of HNO can be explained by its fast conversion into nitric oxide through single-electron oxidation. The action of oxygen on HNO causes a sharp increase in the NO content. In addition, under aerobic conditions, HNO dimerizes (see Scheme 23), and the ratio of the rates of these two processes (oxidation and dimerization) governs the N_2O to NO ratio. Both Piloty´s acid and Angeli´s salt absorb oxygen in a phosphate buffer at pH 7.4 owing to HNO evolution (Scheme 28).

Scheme 28

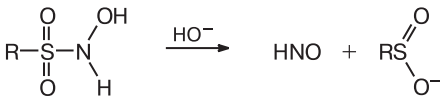


It was shown that under both aerobic and anaerobic conditions, HNO reacts with the superoxide dismutase (SOD), metalloprotein enzyme, with release of NO. Since SOD is a Cu^{2+}-containing enzyme (zinc-, iron-, and copper-containing SOD modifications are also known; here we are speaking about the last-mentioned one), the reaction of HNO with copper(II) acetate was studied, which showed that HNO is rapidly converted into NO under these conditions. Nitric oxide is also formed on treatment of HNO obtained from Piloty´s acid or Angeli´s salt with methemoglobin or other ferroproteins. The action of flavin adenine dinucleotide (but not $NADPH^+$) on HNO gives rise to NO, because flavins, unlike nicotine adenine dinucleotide, can be involved into single-electron transfer. It is of interest that SOD increases the vasodilatory effect of Angeli´s salt by a factor of 30, while SNAP, only twofold, *i.e.*, the action of SOD as an agent converting HNO into nitric oxide is much stronger than the effect exerted on the transformation of NO itself. Conditions of transformations of Piloty´s acid and Angeli´s salt into HNO have been compared.[87] It was shown that Angeli´s salt is converted into HNO at pH 4—8 and that the half-life of HNO amounts to ~20 min at 20 °C (Scheme 29).

Scheme 29



However, Piloty´s acid and its methyl analog require the presence of bases for the HNO release.

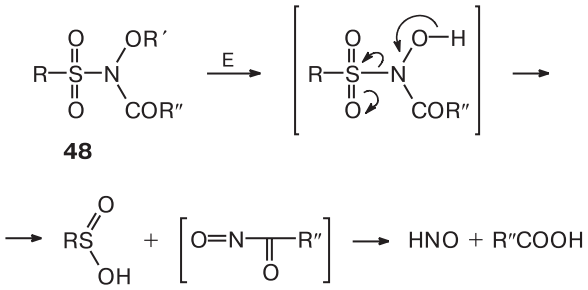


R = Ph, Me

A number of *O*-alkyl-*N*-acyl-*N*-sulfonylhydroxylamine derivatives (**48**) have been studied; it was shown that following enzymatic activation, these compounds undergo a cascade of transformations giving rise to nitroxyl[87,88] (Scheme 30).

In the same study, it was shown that nitrosation of sulfohydroxamic acids gives rise to nitric oxide (Scheme 31).

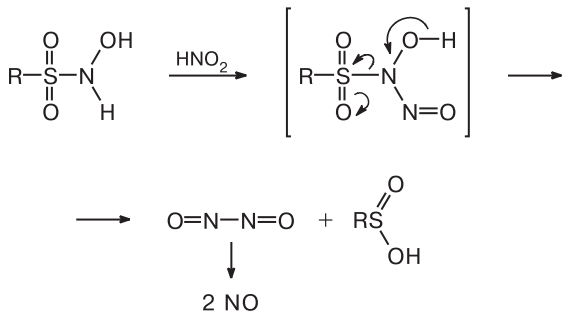
Scheme 30



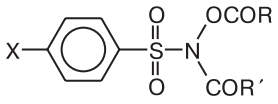
48

E is enzyme.

Scheme 31



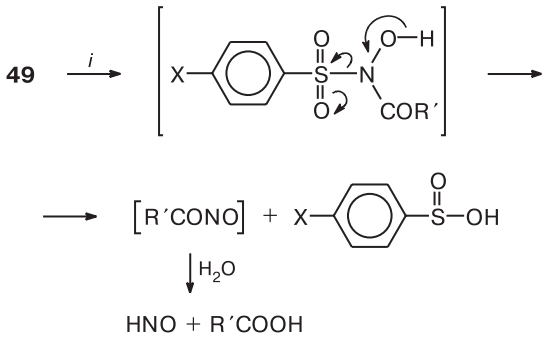
Nitric oxide is reduced by mitochondrial cytochrome *c* to HNO.[89] Nitroxyl readily reacts with biologically important thiols, *e.g.,* glutathione and *N*-acetyl-L-cysteine and enzymes with SH groups in the active site (such as aldehyde dehydrogenase) to give *N*-hydroxysulfenamides. A group of compounds with structure **49** were synthesized[90] as putative prodrugs capable of releasing HNO.



49

Enzymatic or alkaline hydrolysis affords nitroxyl in accordance with Scheme 32.

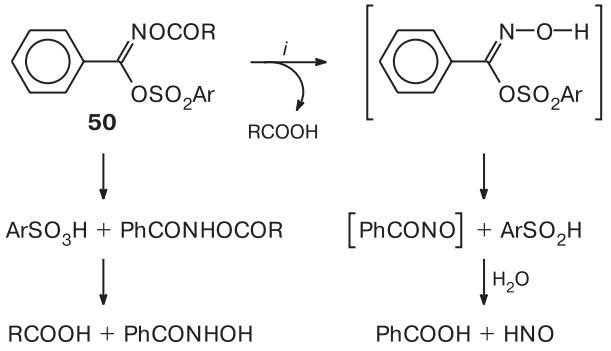
Scheme 32



i. Esterase or HO^-.

Compounds **50**, whose structure and decomposition are presented below (Scheme 33), were reported in the same publication.

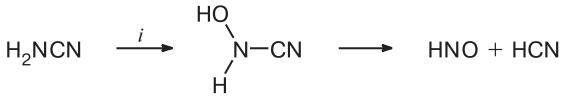
Scheme 33



i. Esterase or HO^-.

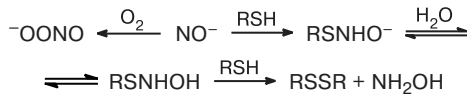
Cyanamide is converted into HNO *via* *N*-hydroxy derivative[88] (Scheme 34).

Scheme 34



i. Catalase, H_2O_2.

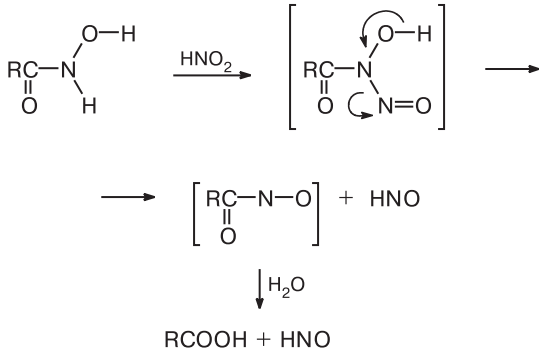
To conclude the discussion of properties and transformations of nitroxyl, we would like to note that this nitric oxide donor has pK_a 4.6, *i.e.*, this acid is almost entirely dissociated at physiological pH values. The NO^- anion present in solution reacts with oxygen to give peroxynitrite and can be reduced by thiols to hydroxylamine with concomitant formation of disulfides.[91]



Piloty´s acid, which is the sulfohydroxamic acid, generates NO upon nitrosation. Under these conditions, hydroxamic acids afford nitroxyl according to Scheme 35.[87]

Hydroxamic acids are fairly attractive as potential NO donors because of their certain resemblance to the part of *N*-hydroxyguanidines (in particular,

Scheme 35



N-hydroxyarginine) that is responsible for nitric oxide release.

A comparison of the activities of a number of oximes (**51**, **52**), hydroxamic acids (**53**), and amide oximes (**54**) as NO generators under conditions of their chemical oxidation has been reported.[92]

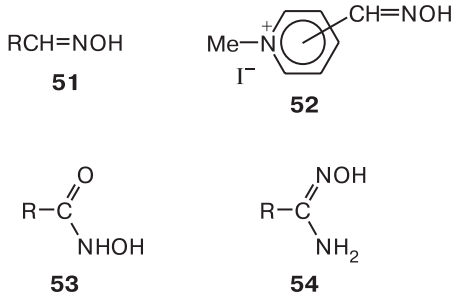


Table 1 presents the results of oxidation of these compounds by potassium ferricyanide in 4% aqueous ethanol at pH 12; the initial concentration of the compounds under study was $2 \cdot 10^{-4}$ mol L^{-1}. Under these conditions, the amount of NO formed from hydroxylamine was 55.8%.

Oximes **51**, **52** did not function as nitric oxide donors under these conditions, which contradicts the data ob-

Table 1. Yield of NO upon the oxidation of compounds **51—54**

R	Yield of NO (%)			
	51	**52**	**53**	**54**
Me	0.0	—	22.9±0.5	25.0±0.5
Ph	0.5±0.1	—	9.00±0.1	10.0±0.2
2-HOC_6H_4	0.0	—	14.0±0.2	—
2-$MeOC_6H_4$	0.0	—	5.1±0.1	—
4-HOC_6H_4	0.0	—	6.5±0.1	—
2-$MeOC_6H_4$	0.0	—	16.0±0.5	—
4-$HOC_6H_4CH_2$	—	—	—	5.5±0.1
2-Py	0.0	6.4±0.1	0.0	21.0±0.2
4-Py	7.4±0.1	6.2±0.1	0.8±0.05	30.0±1

tained for some other oximes (see below). Hydroxamic acids **53**, which are ionized almost completely under experimental conditions (pK_a 8.75—9.46), are much more potent NO donors, *i.e.*, the conjugation of the hydroxylamine fragment with the carbonyl group results in a higher degree of nitric oxide release.

In the case of amide oximes **54**, a sharp increase in the yield of NO was observed upon replacement of the phenyl substituent by the pyridyl group. Pathways of nitric oxide release from oximes and hydroxamic acids were proposed (Schemes 36, 37).

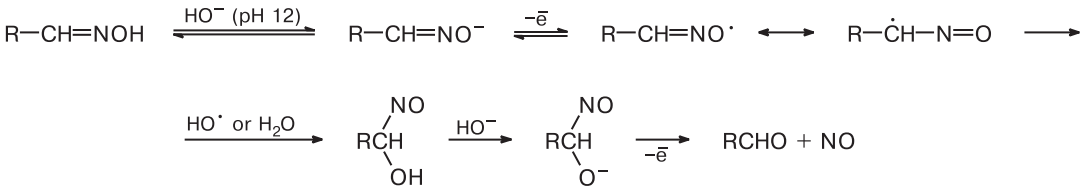
The situation with amide oximes **54** is more complex. It was shown experimentally that the corresponding carboxylic acids, amides, and nitriles are formed under the chosen conditions as the reaction products. This was taken into account when constructing the following scheme for the oxidation of these compounds (Scheme 38).

Data on the activation of sGC were obtained for the hydroxylamine derivatives studied; this confirms that nitric oxide is released under the chosen conditions. The results of this biochemical assay are summarized in Table 2.

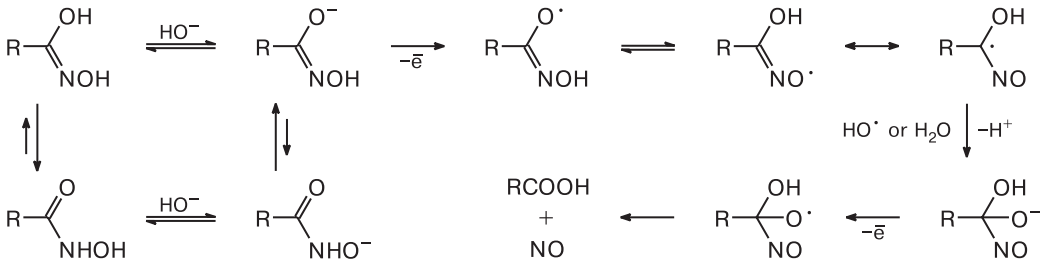
As opposed to oximes that were reported[92] to be very weak nitric oxide donors, oximes derived from quinuclidin-3-one and related compounds proved to act as NO generators upon oxidation, activate sGC, and provide a dose-dependent decrease in blood pressure in narcotized rats when injected intravenously.[93,94] It was suggested[92] that the markedly higher activity of quinuclidin-3-one oximes is due to their rigid structure, which provides the possibility of intramolecular interaction of adjacent groups with the oxime fragment. Compounds **55—61** of this series were studied.

All these compounds generate NO but the highest activity was found for those substances whose molecules contain an *ortho*-hydroxy group located close to the oxime fragment. A probable reason is the fact that this functional group can stabilize the nitric oxide evolved (for

Scheme 36



Scheme 37



Scheme 38

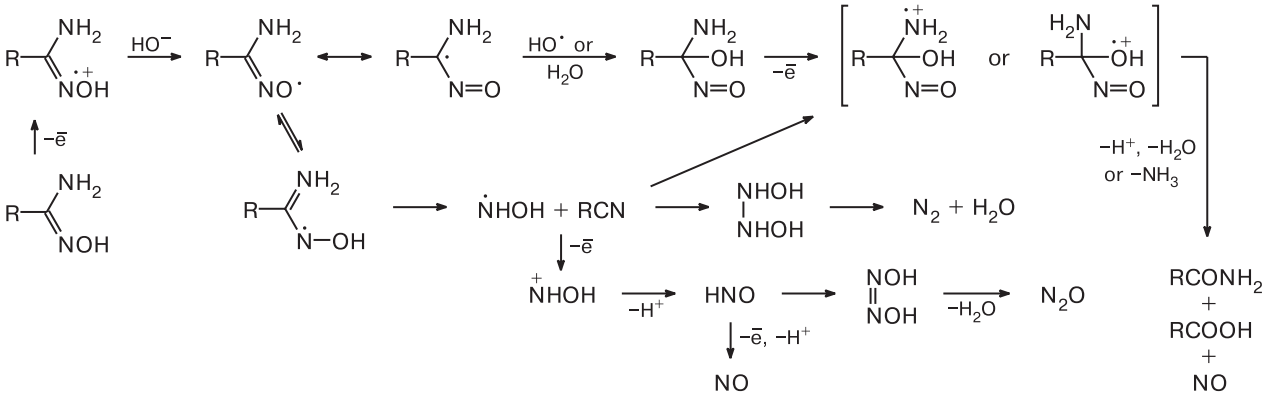
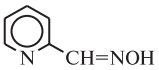
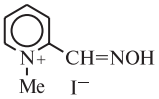
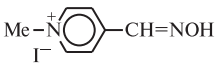
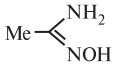
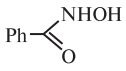
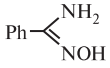


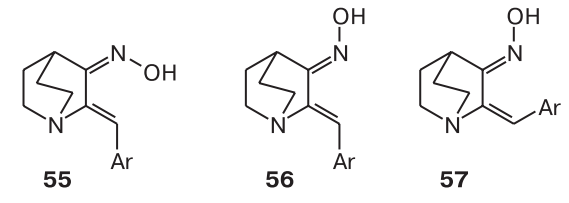
Table 2. Activation of soluble guanylate cyclase (sGC) by hydroxylamine derivatives

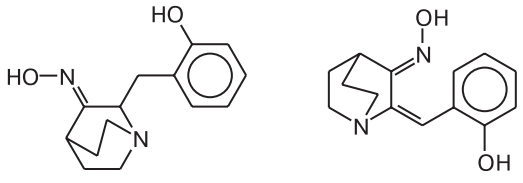
Compound	*a*/act.u. at *C**/mol L^{-1}			
	10^{-6}	10^{-5}	10^{-4}	10^{-3}
	1.3±0.1	2.0±0.1	1.6±0.1	—
	1.4±0.1	2.1±0.1	1.1±0.1	—
	1.1±0.1	1.6±0.1	1.5±0.1	—
	—	1.4±0.1	1.3±0.1	1.3±0.1
	—	1.4±0.1	1.7±0.1	1.5±0.1
	—	1.4±0.1	1.3±0.1	1.2±0.1
$NH_2OH \cdot HCl$	1.1±0.1	2.5±0.1	2.2±0.1	3.6±0.1

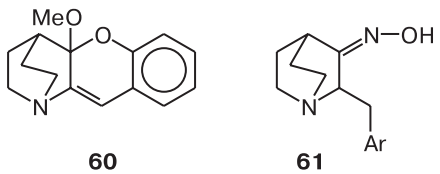
Note. The initial activity was taken to be unity.
* The concentration of the hydroxylamine derivative.

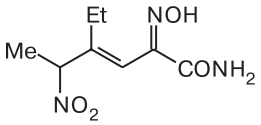
example, by forming nitrites) and function as an intramolecular carrier.

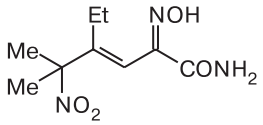
Of the NO donors that contain an oxime group and exhibit a substantial biological activity, noteworthy are (3*E*)-4-ethyl-2-hydroxyimino-5-nitrohex-3-enamide (FK-409), which is now under clinical research,[95] and structurally related compounds **62** and **63**.[96,97]

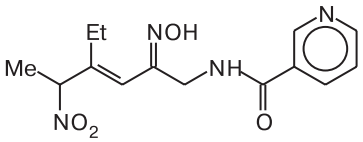

55 **56** **57**


58 **59**


60 **61**


FK-409


62


63

Pharmacological properties of these compounds are typical of nitric oxide donors. They are regarded as promising antianginal drugs; they inhibit platelet aggregation and exhibit a vasodilatory effect. It is important that FK-409 displays the activity without thiol groups, which distinguishes it from nitrovasodilators.

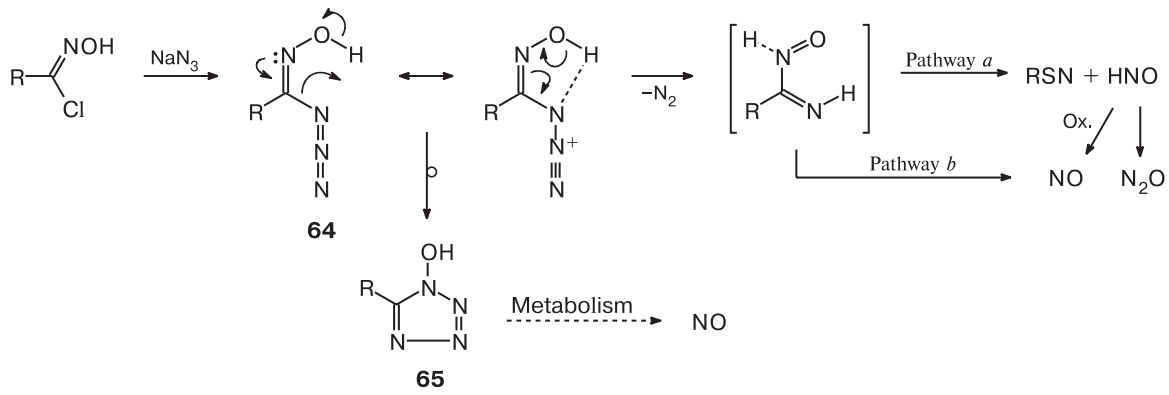
It was found that enhanced ability to generate nitric oxide is observed in those cases where electron-withdrawing substituents are present in the geminal position to the oxime group. For example, nitrolic acids $RC(NO_2)=NOH$ decompose to give HNO even at room temperature.[98]

The properties of a series of azide oximes (**64**) and 1-hydroxytetrazoles **65**, prepared from **64**, have been studied. Both groups of compounds were found to be potent inhibitors of thrombus formation and to decrease blood pressure, the activity of azide oximes being substantially higher.[99] It was found by a chemiluminescence technique that these compounds are NO donors under aerobic conditions. A pathway from these compounds to nitric oxide has been proposed[99] (Scheme 39).

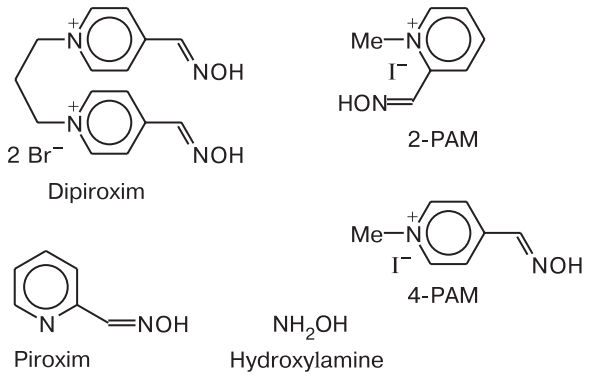
Destruction under anaerobic conditions was shown to give only N_2O (note for comparison that SIN-2 produces 0.03% N_2O and 2.73% NO under these conditions). Presumably, the sharp increase in the production of NO under aerobic conditions shows that pathway (*a*), which is effective in the absence of oxygen, becomes less significant, and homolytic cleavage of the C—NO bond and pathway (*b*) giving NO rather than HNO become the principal route of decomposition of these compounds.

Yet another important point is the problem of reactivation of choline esterase (CE), the enzyme that catalyzes hydrolysis of acetylcholine (AC). After this mediator has entered the synaptic gap, its excess amount should be eliminated, otherwise further conduction of a nerve impulse is impossible.[41] The whole cholinergic inervation is determined by the activity of this enzyme. Organophosphorus inhibitors of choline esterase phosphorylate the hydroxy group of serine in the enzyme, but this reaction is almost irreversible (unlike acetylation of choline).

Scheme 39



This results in CE inhibition; as a consequence, organophosphorus military poison gases and many insecticides can cause poisoning with fatal outcome. This stipulates the insistent need for compounds capable of dephosphorylating the serine residue and restoring the CE activity. The following compounds are used for this purpose:



The main idea of using these compounds was as follows: due to the presence of the oxime fragment in the molecule, they are able to attack the phosphorus—oxygen bond as nucleophiles and to dephosphorylate the deactivated enzyme. This mechanism appears to be realistic, but recently a different idea has been put forward to interpret the action of these compounds.[100] It can be seen from the above structural formulas that choline esterase is reactivated by hydroxylamine or oximes, *i.e.*, by compounds able to generate nitric oxide. The function of these compounds as NO donors is due to sGC activation followed by an increase in the level of cGMP, which results, in particular, in dephosphorylation of phosphates through activation of the cGMP-dependent protein kinase* and Ca^{2+}-ATPase. Experimental verification of these assumptions showed that each of these reactivators actually stimulates sGC (Table 3).

Table 3. Activation of sGC (*a*) by choline esterase reactivators at various concentrations (*C*) of the studied compounds*

Compound	*a*/act.u. at *C**/mol L^{-1}			
	10^{-6}	10^{-5}	10^{-4}	10^{-3}
Dipiroxim	1.50±0.09	2.00±0.09	3.20±0.15	4.20±0.13
Hydroxylamine	1.10±0.02	1.30±0.05	2.10±0.1	3.60±0.14
Piroxim	1.40±0.05	1.95±0.1	1.70±0.03	—
2-PAM	1.20±0.06	2.10±0.05	1.70±0.05	—
4-PAM	1.10±0.02	1.60±0.05	1.50±0.04	—

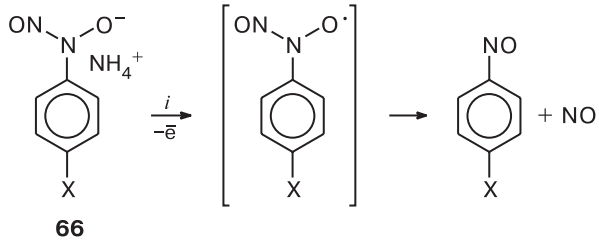
* The initial activity was taken to be unity.

Thus, the new approach to the development of effective choline esterase reactivators deserves attention.

4.7. *N*-Nitroso derivatives

It has been found[101] that electrooxidation of ammonium salts of *p*-substituted *N*-nitrosohydroxylamines (**66**) yields nitric oxide and the corresponding nitrosobenzenes (Scheme 40).

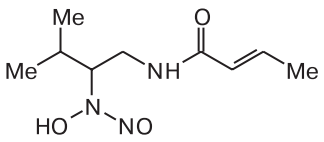
Scheme 40



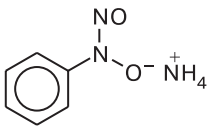
i. Electrooxidation.

The *N*-nitroso-*N*-hydroxyamine fragment is a part of a known dopamine-β-hydroxylase inhibitor, dopastin, and a complexing agent, Cupferron.

* The cGMP-dependent protein kinase is an enzyme that catalyzes the transfer of phosphate groups during chemical reactions.



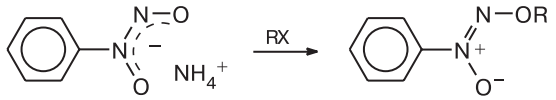
Dopastin



Cupferron

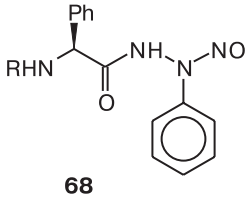
Transformation of *N*-nitroso-*N*-hydroxyaniline derivatives with release of nitric oxide can be induced not only by electrooxidation[101] but also by horse-radish peroxidase.[102] It was shown that Cupferron and its analogs undergo selective alkylation at the terminal oxygen atom to give alkoxydiazene oxides **67** (Scheme 41).

Scheme 41

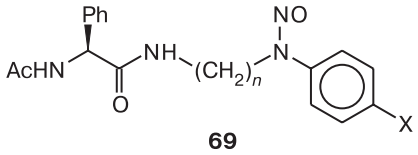


67

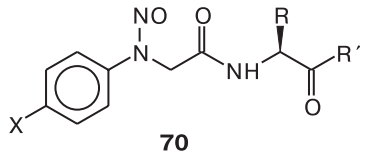
The obtained compounds are stable under ordinary conditions but release nitric oxide either photochemically or enzymatically (under the action of α-chymotrypsin) and are considered to be promising prodrugs.[103] A number of *N*-nitroso derivatives (**68—70**) without the *N*-hydroxy group were also synthesized and shown to perform *trans*-nitrosation.[104] It was found that compounds of this type are efficient cysteine proteinase inhibitors.



68



69



70

68: R = Ac, AcAla

69: *n* = 2, X = OH; *n* = 3, X = Cl, OH; *n* = 4, X = OH

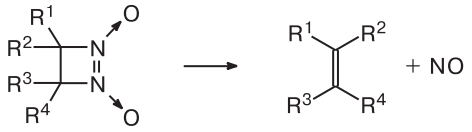
70: X = OH, R = Bu^t or PhCH$_2$, R´ = OMe;
X = H, R = Me, R´ = OMe;
X = H, R = Bu^t, R´ = PheOMe

Treatment of papain with these compounds results in its *S*-nitrosation.

4.8. *N*-Oxides

This section will be started with discussion of the studies concerning electrochemical, pharmacological, and biochemical investigations into 1,2-diazetine 1,2-dioxides (**71**), a new class of nitric oxide donors, possessing vasodilatory activity[105,106] (Scheme 42). According to electrochemical detection, all these compounds generate nitric oxide.

Scheme 42



71a—g

a: R^1 = Et, R^2 = Br, R^3 = R^4 = Me;
b: R^1 = R^3 = R^4 = Me, R^2 = Br;
c: R^1 = Br, R^2 = Ph, R^3 = R^4 = Me;
d: R^{1}R^3 = (CH$_2$)$_4$, R^2 = Br, R^4 = Me;
e: R^{1}R^3 = (CH$_2$)$_5$, R^2 = R^4 = H;
f: R^1 = H, R^2 = Ph, R^3 = R^4 = Me;
g: R^1 = H, R^2 = R^3 = R^4 = Me

The degree of NO generation depends on the structure changes in the sequence **e** < **g** < **a** < **c** < **b** < **f** < **d**.

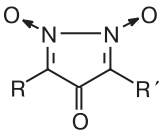
In terms of sGC activation, compounds can be arranged in the series **e** < **g** < **a** ≈ **c** < **b** ≈ **f** < **d**.

In terms of the antispasmodic effect (for amounts of introduced substances varying from 10^{-6} to 10^{-11} g mL^{-1}), the following sequence was obtained: **e** < **g** < **a** < **f** < < **c** < **b** < **d**.

Finally, the sequence based on the hypotensive activity was as follows: **e** < **g** < **a** < **b** < **f** < **c** < **d**.

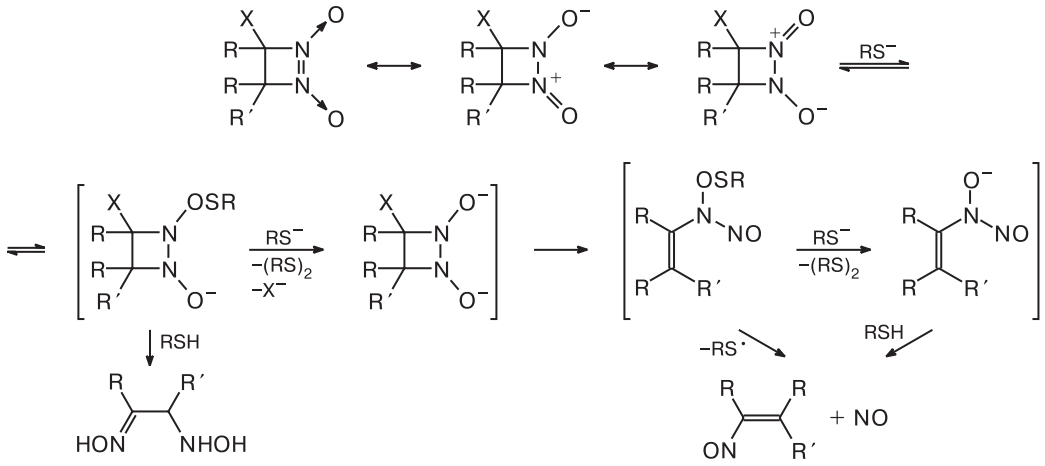
As can be seen from the above data, there exists remarkably good agreement between the level of nitric oxide generation by the derivatives in question, the sGC activation by these compounds, and their antispasmodic and hypotensive activities. According to all types of assay, compound **71d** is the most active. In addition to the above-noted types of activity, these compounds inhibit platelet aggregation.[107,108] The kinetics of nitric oxide release during biotransformation of these derivatives *in vitro* and *in vivo* have been measured.[109] It has been found[110] that the formation of nitric oxide upon their degradation is sharply accelerated in the presence of, for example, cysteine and glutathione. The reactions involved[110] are shown in Scheme 43.

N,*N*´´-Dioxides of the pyrazole series (**72**), which exhibited antithrombotic properties due to release of nitric oxide, bear some structural resemblance to the 1,2-dioxides considered above.[111,112]

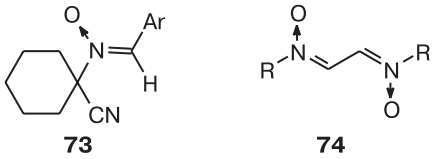


72

Scheme 43



In view of the fact that only compounds in this series containing electron-withdrawing substituents did exhibit pronounced activity, nitrones **73** with a cyano group in the geminal position and a group of glyoxal nitrones (**74**) have been synthesized.[113]

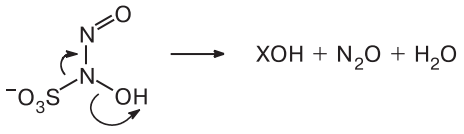


73 **74**

Compounds of both types exhibited antithrombotic effect. It was shown that they release only a small amount of nitric oxide under the action of an Fe^{3+}-porphyrin complex and an oxidant. However, the antithrombotic effect *in vivo* is pronounced. Presumably, some additional factors are effective *in vivo* to ensure a higher level of NO release.[113]

Compounds in which the NONO fragment is connected to a sulfo rather than to an amino group release nitrous oxide N_2O instead of nitric oxide (Scheme 44).[114]

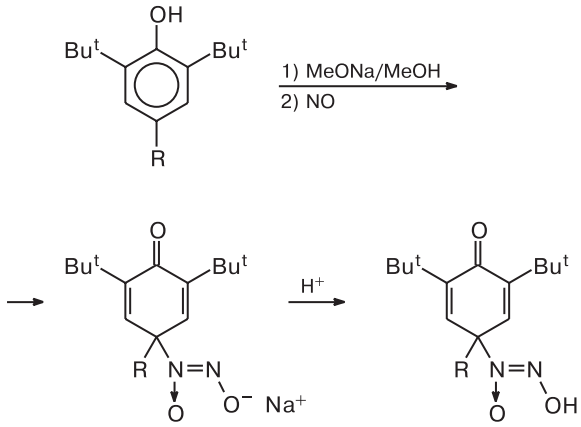
Scheme 44



4.9. Diazenium-1,2-diolates (NONOates)

It has been shown[115] that nitric oxide reacts with phenols containing bulky groups in the *ortho*-positions (typical structures of antioxidants) giving rise to diazenium-1,2-diolates **75**, which were reasonably considered to be potential nitric oxide donors (Scheme 45).

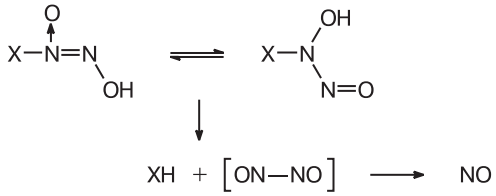
Scheme 45



75

The properties of this type of compound have been considered previously;[4] however, numerous publications devoted to the chemistry of these, quite promising nitric oxide donors have appeared in recent years. Diazenium-1,2-diolates are compounds that generate nitric oxide at physiological pH values without enzymes, the rate of NO release being determined by the nature of the leaving group X [11,87] (Scheme 46).

Scheme 46



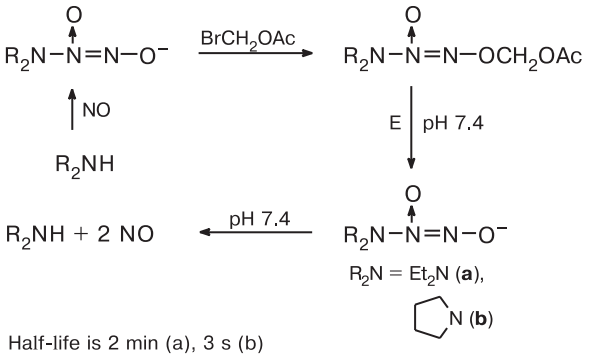
Evolution of nitric oxide is also possible as a result of enzymatic activation of diazenium-1,2-diolates,[87,116] al-

though these compounds also undergo hydrolysis without enzymes[116] (Scheme 47).

Scheme 47

Recently, a large group of compounds of this class requiring enzymatic cleavage of the esterase groups have been prepared. These are unique diazenium-1,2-diolates that do not undergo spontaneous hydrolysis in an aqueous medium. When these substances are added to leukemic cells, a substantial amount of nitric oxide is released, *i.e.*, intracellular activation of NO formation takes place. This is accompanied by inhibition of the leukemic cells, which includes the development of apoptosis* (Scheme 48).

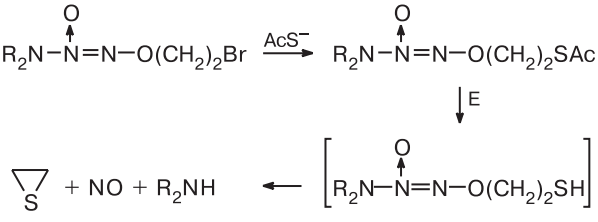
Scheme 48



E is esterase.

A similar type of cleavage was also found for acetylthioethyl derivatives of diazenium-1,2-diolates[117] (Scheme 49).

Scheme 49



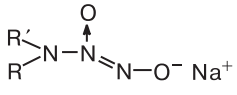
E is esterase.

* Apoptosis is the programmed cell death.

Study of this approach is quite topical because the involvement of endogenous thiols in metabolism is very significant.

Mention should be made of an important conclusion drawn in the study cited:[117] the main obstacle hampering the use of nitric oxide generators in the therapy of tumor diseases (and many other pathologies) is related to the action of NO on other targets, especially on the cardiovascular system.

Study of donor compounds capable of spontaneous generation of nitric oxide led to the conclusion that several diazenium-1,2-diolates are promising for clinical trial as vasodilatory drugs, platelet aggregation inhibitors, and cytostatics. These compounds were shown to be fairly active both *in vitro* and *in vivo*. Diazenium-1,2-diolates **76** were synthesized and studied.[118]



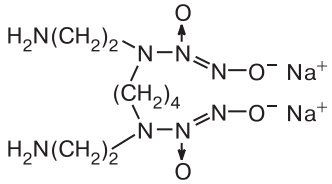
76

R = R´ = Et
R = R´ = $(CH_2)_2NH_2$
R = R´ = $(CH_2)_3NH_2$
R = $(CH_2)_3NH_2$,
R´ = $(CH_2)_4N^+H_2(CH_2)_3NH_2$
R = Me, R´ = $(CH_2)_6N^+H_2Me$
R = Pr, R´ = $(CH_2)_3N^+H_3$

In the authors´ opinion, two factors should be taken into account when planning research in this field. First, the rate of dissociation of the compound to give nitric oxide is important. The regard for this rate is a standard requirement for NO donors because it is necessary to find the optimum time of NO formation in each particular case. Second, one must know the toxicological properties of products that are formed together with nitric oxide. Indeed, cleavage of compound **76** (R = R´ = Et) gives *N*-nitrosodimethylamine, which is a potential carcinogen.

Diazenium-1,2-diolates can also be used for purposes unusual for NO donors. For example, intratracheal administration of diazenium-1,2-diolates together with surfactants was studied and this was shown to improve oxygen saturation of tissues and to decrease pulmonary hypertension.[119]

Although the key effect of nitric oxide is related to sGC activation and to an increase in the cGMP content in organs and tissues, it has been shown recently that this is not the only mechanism of NO action. Study of spermine diazenium diolate (**77**), a representative of the

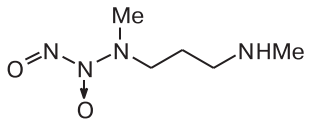


77

diazenium-1,2-diolate group, showed that vessel relaxation is largely retained upon the addition of an sGC inhibitor (ODQ, see Section 4.5).[120]

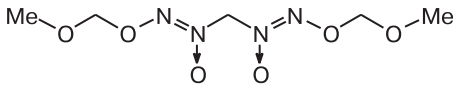
In other words, relaxation caused by this nitric oxide donor is not affected by ODQ. Most likely, this is due to the fact that the action of these substances is due to not only sGC activation, but also depends on other factors. Presumably,[120] vessel relaxation can also be induced without action on sGC, *viz.*, by activation of potassium channels in the cell membrane and the enzymes (Na^+/K^+ ATP-ase and Ca^{2+}-ATP-ase) of the endoplasmic reticulum.* According to the researchers´ hypothesis,[120] which requires experimental proof, activation of potassium channels is the most probable reason for the vasodilatory action of diazenium-1,2-diolates.

Currently, a series of diazenium-1,2-diolates are under research. Thus the compound NOC-7 is studied in relation to the hemodynamic effect it induces, similar to the effect of sodium nitroprusside. This compound is an ultrashort-term nitric oxide donor, which suppresses the dose-dependent effect on the blood pressure reduction[121].



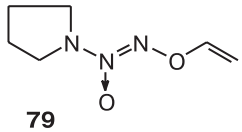
NOC-7

Compound **78** is a potential cytostatic for the therapy of leukemia; it inhibits the growth of murine leukemia P385 by 85%, whereas the known drug used for this purpose, *N*-methyl-*N*-nitrosourea, provides inhibition by only 35%.[122]



78

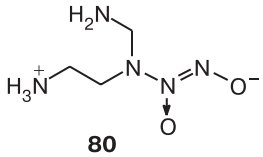
Alkoxydiazene oxide **79**, containing a pyrrolidine ring as the substituent, is meant for the use as a hepatoprotector.[123]



79

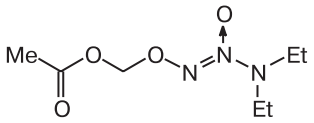
It is important that this compound is selectively accumulated in liver.

Compound **80** is a potential neuroprotector for the treatment of parkinsonism.[124]

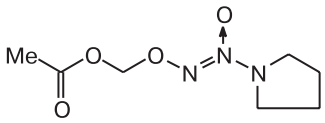


80

Compounds **81** and **82**, which release large amounts of nitric oxide in leukemic NO-sensitive human cells, efficiently inhibit tumor growth and induce apoptosis of these cells.[117,125]



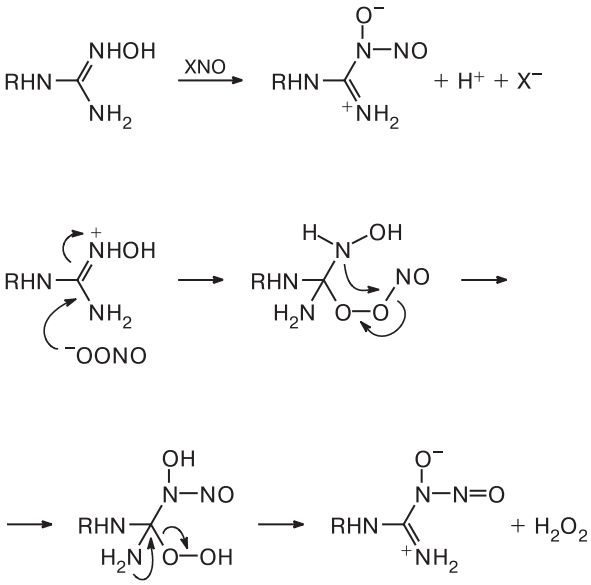
81



82

A large group of compounds containing guanidine fragments have been synthesized[126] (Scheme 50).

Scheme 50

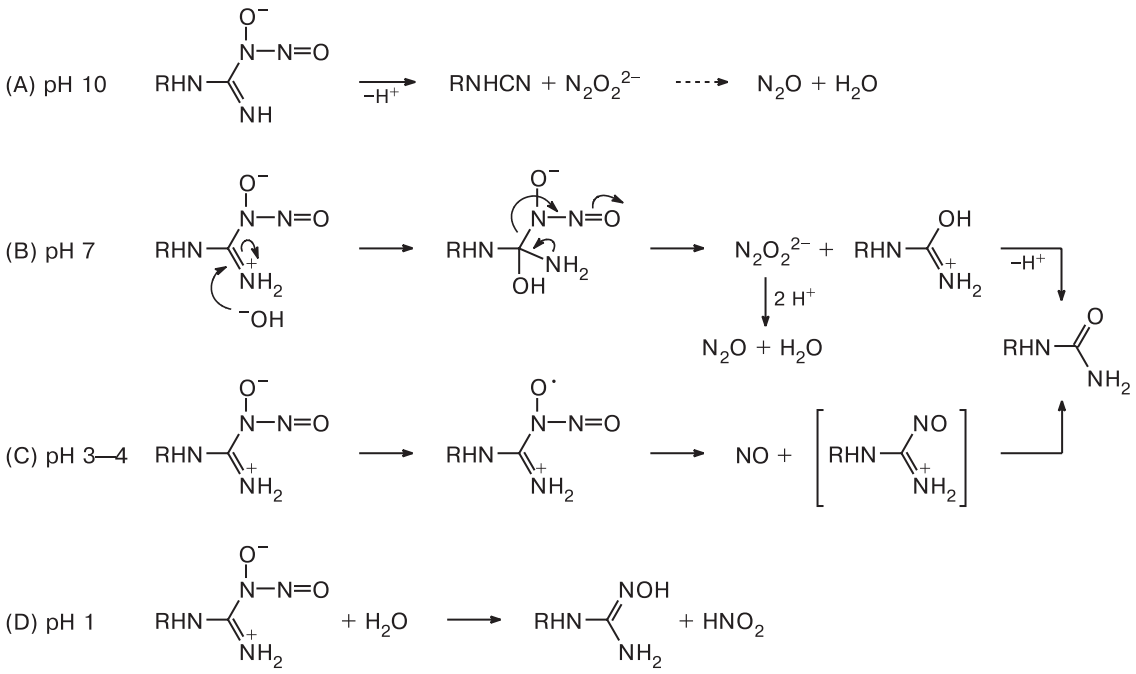


The decomposition pathways for this type of compound were found to be markedly dependent on the solution pH (Scheme 51).

The evolution of nitric oxide is responsible for the vasorelaxant properties of hydroxyguanidines. The possibility of these transformations points to other ways of

* The endoplasmic reticulum is a cellular structure representing a system of tubules that ensure the transport of substances in the cell cytoplasm of the lateral-muscle fibers.

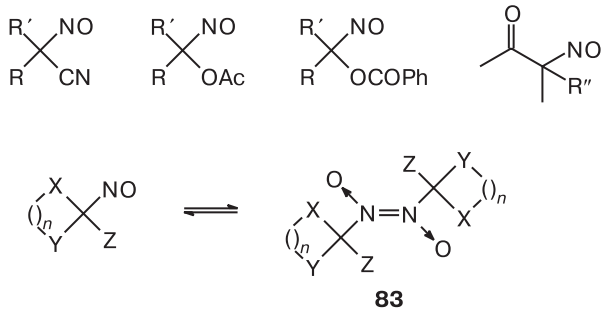
Scheme 51



nitric oxide formation, which may be also effective *in vivo*. The use of inducible nitric oxide synthase (iNOS) is known to give not only NO but also N_2O, pointing to the possibility of *N*-nitrosation, yielding diazenium-1,2-diolates, in particular, under physiological conditions.

4.10. *C*-Nitroso and *C*-nitro derivatives

A number of publications[127—129] report the synthesis of *C*-nitroso compounds activated by electron-withdrawing groups in geminal and vicinal positions. Cases where a cyano group was in the geminal position with respect to the NO-donor fragment have already been mentioned[83,113] (see Sections 4.6 and 4.8). These *C*-nitroso derivatives readily dimerize to give azo dioxides **83**.

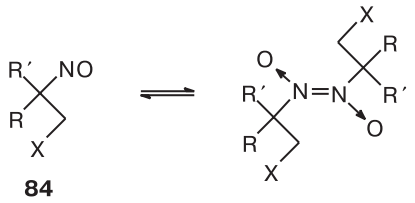


83

X = CH_2, CO; Y = CH_2, OCO, CO;
Z = CN, OAc, Me, OCOR, COOEt, COMe

Active inhibitors of platelet aggregation have been found in this series, cyano derivatives being the most active. It was found that these compounds decompose in the aqueous medium at 37 °C to give NO and HNO.[127]

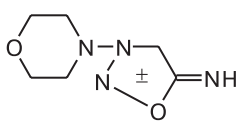
Yet another group of substances that also exhibit antithrombotic action and inhibit platelet aggregation are *C*-nitroso compounds **84**, which dimerize to give azo dioxides.[128] These compounds decompose in ethanol to give nitric oxide and nitrous oxide (N_2O is formed *via* HNO).



84

X = NO_2, COMe, COOH, OAc

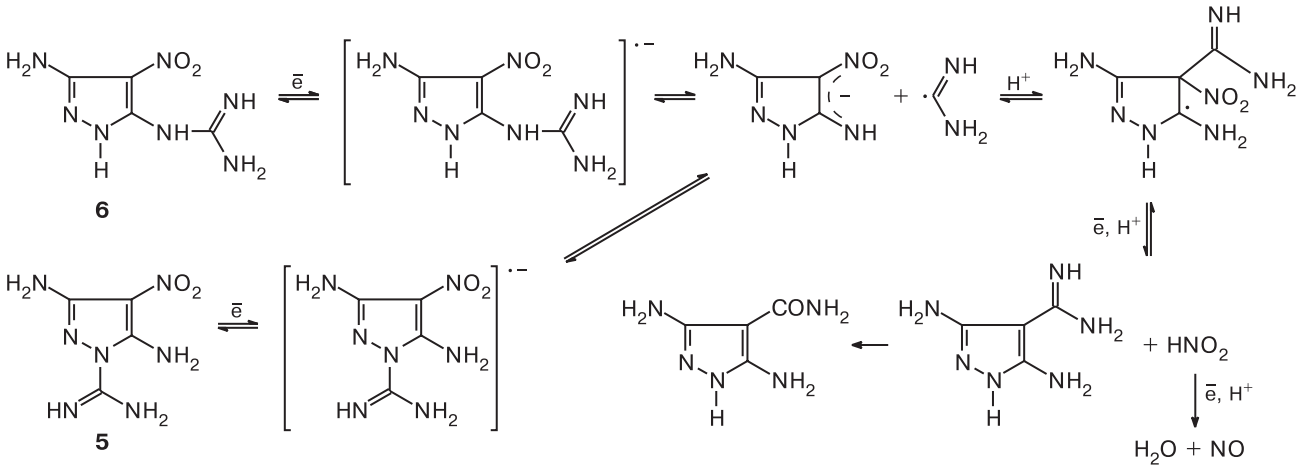
Compound **84** (R = H, R´ = Ph, X = NO_2) forms NO (0.18%) and N_2O (3.81%). Note for comparison that SIN-1 (the key metabolite of molsidomine) forms 0.9% nitric oxide and no N_2O under these conditions.



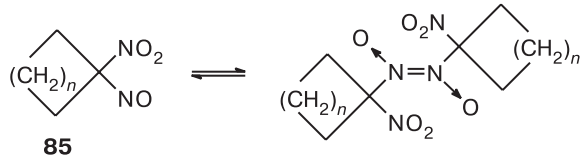
SIN-1

Yet another study by the same authors[129] is devoted to nitro nitroso compounds **85** (pseudonitroles), which

Scheme 52



tend to evolve NO and N_2O in an aqueous medium at 37 °C.



85

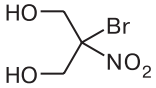
Compounds **85** synthesized inhibit platelet aggregation. Degradation of these compounds gives nitric oxide. The researchers point out that the absence of influence on the blood pressure suggests that enzymatic rather than thermal decomposition is effective *in vivo*.

(3*E*)-4-Ethyl-2-hydroxyimino-5-nitrohex-3-enamide (FK-409), which has already been mentioned, is now under phase II clinical trial as a thrombus formation inhibitor [130] (see Section 4.6).

In this compound, two functional groups, the oxime and aliphatic nitro group, may be responsible for the nitric oxide release; however, this aspect is not clear yet (see Section 4.6).

Nitropyrazole derivatives **5** and **6** generate[25] nitric oxide not only upon oxidation but also upon reduction. The hypothetical pathway to NO upon reduction of these compounds is shown in Scheme 52.

The effect of the well-known antiseptic bronopol (3-bromo-3-nitropropylene glycol)[131] is explained by the fact that, owing to the presence of the activated C—Br bond, this compound can cross-link the sulfhydryl groups of dehydrogenases to give disulfides. This results in modification of the microbial cell walls and disturbs their metabolism.

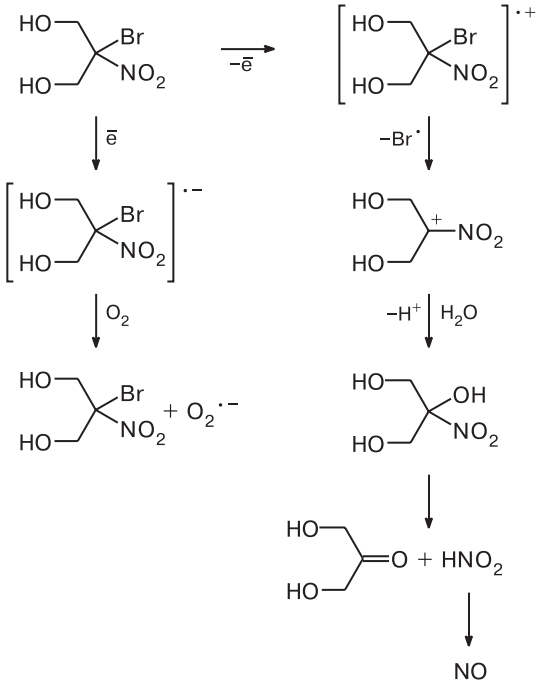


Bronopol

Elimination of formaldehyde caused by the presence of two hydroxymethyl groups also contributes to the antimicrobial action of this compound.

Another possible mechanism of the biological action of bronopol is attributed[131] to the release of nitric oxide and to oxidation of bronopol radical anion to give superoxide radical anion (Scheme 53).

Scheme 53



Further processes are related to the formation of NO and peroxynitrite $ONOO^-$.

Decomposition of bronopol in the absence of oxygen and in the presence of oxidants such as hydrogen peroxide or potassium ferricyanide was also studied. Nitric oxide was quantified by polarography based on the yield of nitroprusside anions.

Under oxidative conditions, bronopol is an efficient nitric oxide donor (nitroethane also was shown to generate NO, although to a lesser extent). Its capacity for sGC activation, typical of NO donors (Table 4), was studied.

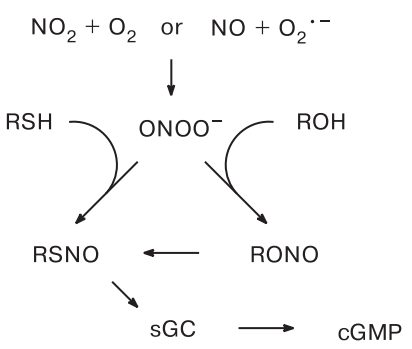
C* /mol L^{-1}	10^{-6}	10^{-5}	10^{-4}
Activation a/act.u. (%)	2.6 (260)	4.6 (460)	0.8 (80)**

Note. The initial activity of sGC was taken to be unity.
* The bronopol concentration.
** Inhibition.

When the bronopol concentration increases, activation is replaced by retardation, possibly, due to the oxidation of the thiol groups of the enzyme by peroxynitrite formed or upon alkylation. It cannot be ruled out that the formation of the strong oxidant, peroxynitrite, leads to the modification of sulfhydryl-containing components of dehydrogenases in microbial cell walls, which ensures the bactericidal effect of bronopol.

It was shown[132] that peroxynitrite has a fairly short half-life in the organism (about 1 s), and the conjugate acid, *i.e.*, peroxynitrous acid, has pK_a 6.8. Peroxynitrite anion is able to oxidize thiols and lipids and to nitrate proteins, carbohydrates, and nucleic acids. In isolated organs, too, peroxynitrite exhibits NO-like effects (blood vessel relaxation, inhibition of platelet aggregation, leucocyte adhesion to endothelium, sGC activation). The presence of peroxynitrite in the organism is considered to be responsible for the formation of nitrotyrosyl residues in proteins, which is considered to be related to various pathological conditions. The formation of stable nitric oxide donors in the reactions of peroxynitrite with glycerol, glucose, or thiols is not tissue-specific. Nitric oxide donors are formed upon the reaction of $ONOO^-$ with blood plasma. The formation and transformations of peroxynitrite is depicted in Scheme 54.

Scheme 54



4.11. Heterocyclic compounds

Here, we shall list the most significant characteristics of the NO concept for prodrugs.[112]

1. It is important to know whether the release of NO is an intra- or extracellular process.

2. An important factor is the duration of NO release; fast-acting compounds are used against acute pathologies, while slowly acting drugs are used for prophylaxis.

3. The chemical and physical properties of the compounds used, which determine, for example, the possibility of intravenous administration, are also important. A significant factor is a sufficient half-life period.

4. An important role is played by the charge of the released NO species (NO^0, NO^+, or NO^-), their interconversions, and the capacity for nitrosation.

5. Co-factors for NO formation, *e.g.*, thiols, oxygen, and the corresponding enzymes, are necessary.

6. Stability of the drugs against light, heating, and the medium pH are important factors.

7. The data on the rate of NO release are required; there is information that toxic effects diminish in the case of slow release.

Other important types of heterocyclic derivatives among which effective nitric oxide donors have been discovered are sydnonimines and other mesoionic compounds.

Exogenous NO donors

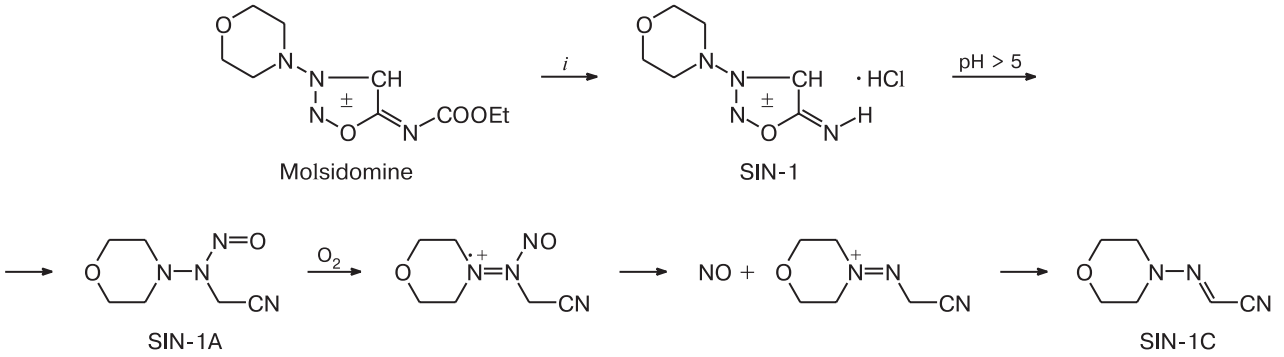
4.11.1. Sydnonimines and related compounds

Decomposition of sydnonimines is sensitive to oxygen and light and gives, in addition to nitric oxide, superoxide radical anion, and, hence, peroxynitrite and hydroxyl radical.[12] The pattern of metabolism and degradation of a well-known antianginal drug, molsidomine, is given as an example[112] (Scheme 55).

It was shown[112] that decomposition of SIN-1A (unlike SIN-1) is a pH-independent process and this stage involves oxygen. There exists a correlation between the consumption of oxygen and the formation of nitric oxide in solutions of SIN-1 with a constant pH. It is significant that it is NO rather than HNO that is formed with SIN-1C from SIN-1A; SIN-1A activates sGC, increases the cGMP concentration in cells, and induces effects typical of nitric oxide donors.[133]

The mechanism of sGC activation under the action of SIN-1 has been studied.[134] It was shown that the level of this activation is determined by the presence of glutathione (GSH) in the medium and by its reactions with nitric oxide and superoxide radical anions. It was found that the resulting peroxynitrite is not an sGC activator. The reaction of glutathione with NO—superoxide systems gives rise to *S*-nitrosoglutathione (GSNO). The

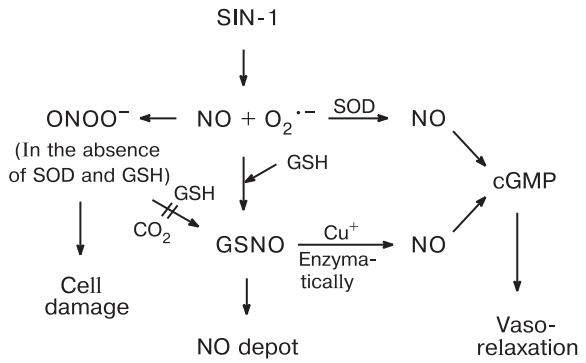
Scheme 55



i. Liver esterases

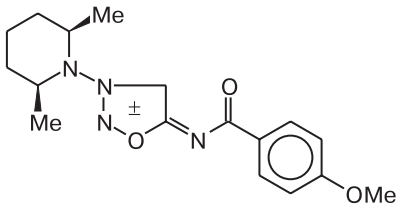
thiyl radical (see Section 4.5) formed upon the oxidation of GSH with oxygen, superoxide, or hydrogen peroxide might react with NO to give GSNO. Cu^+-dependent nitric oxide release is assumed, although S-nitrosoglutathione might also undergo enzymatic cleavage with GSH peroxidase, γ-glutamyl transferase, and thioredoxin reductase, which catalyze the transformation of GSNO into NO (Scheme 56).

Scheme 56

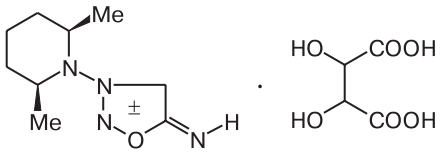


It was found that SIN-1 participates in *S*-nitrosation through the formation of peroxynitrite without activating sGC or increasing the levels of cGMP and cAMP in tissues. It was demonstrated that without these effects, NO donors (this refers not only to SIN-1 but also to diazenium-1,2-diolates) can also stimulate the cardiac muscle contraction. Conversely, sodium nitroprusside stimulates the formation of cGMP but does not produce this effect.[135] The analgesic effect of SIN-1 is definitely related to sGC activation.[136] It is significant that pain relief under the action of SIN-1 depends on the dose; low doses relieve pain, while high doses either do not have any effect or enhance pain. An sGC inhibitor, ODQ (see Section 4.5), eliminates the analgesic effect of low doses and reduces the effect of high doses of SIN-1. Hemoglobin (the "scavenger and destroyer" of nitric oxide) also inhibits the effect of low doses and reduces the action of high doses. It was shown[137] that SIN-1 has an antiulcer activity.

A number of compounds of the sydnonimine series attract attention in relation to their high biological activities. This group comprises molsidomine analogs such as pirsidomine, which is a strong long-acting vasodilator. Like molsidomine, pirsidomine is first metabolized in the organism and the metabiolites act as nitric oxide donors.[138] The key metabolite, darsidomine tartrate, induces selective dilatation of the large coronary artery, exerts an antiischemic action, and causes no addiction when the drug is taken chronically.[139]

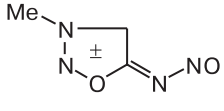


Pirsidomine



Darsidomine tartrate

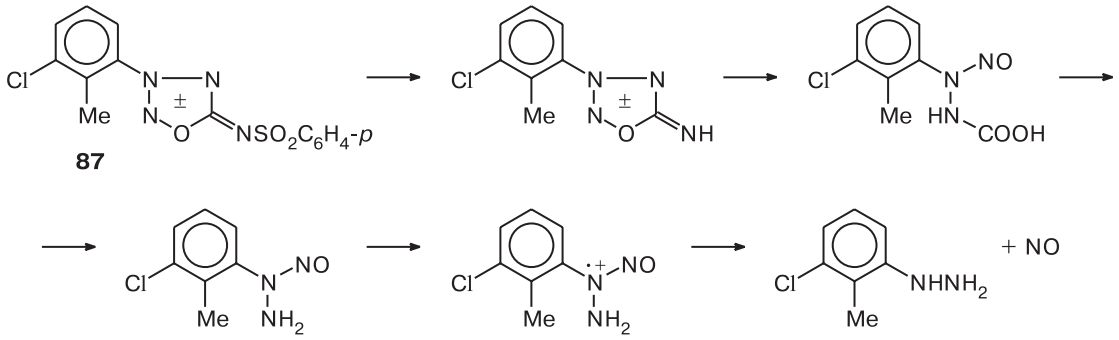
Antithrombotic and vasodilatory properties and the ability to inhibit platelet aggregation was found for an *N-exo*-nitroso derivative of sydnonimine, RE-2047.[140]



RE-2047

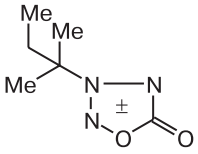
Like sydnonimines, derivatives of other mesoionic compounds exhibit clear-cut properties of nitric oxide donors and

Scheme 57



87

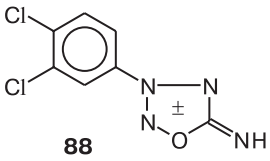
exert the corresponding biological effects. Thus the oxatriazolium derivative **86** was found to be a fairly efficient inhibitor of platelet aggregation inducing stable decrease in the blood pressure in model experiments with animals. However, the development of this drug was terminated due to the discovered mutagenic effect.[112]



86

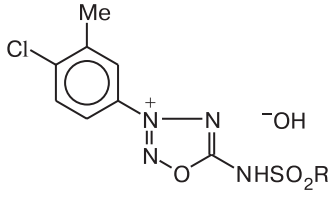
Another oxatriazolium derivative **87** [141] attracts attention because of its action similar to the action of sodium nitroprusside but without evolution of cyanide and, correspondingly, without the hazard of potential toxicity. This compound was recommended for use at hypertensic crises.[141] The mechanism of action of this compound[112] includes either hydrolytic or enzymatic ring opening to give an unstable carbamate, its spontaneous decarboxylation to give nitrosohydrazine, which eliminates nitric oxide upon oxidation by oxygen, especially in the presence of traces of transition metal ions such as iron ions (Scheme 57).

Compound **88** has an antithrombotic and an antiasthmatic effects; it is a potent inhibitor of ADP-induced platelet aggregation and sGC activator, which increases the level cGMP.[142]



88

Two mesoionic compounds of the type **89** that function as NO donors with a bronchodilatory action are intended for use as antiasthmatic drugs.



89

R = Me, 4-$MeOC_6H_4$

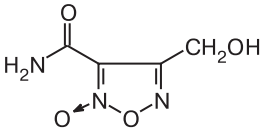
4.11.2. Furoxanes

Furoxanes represent one more class of heterocyclic compounds whose derivatives act as nitric oxide donors and which therefore attract substantial attention of pharmacologists, biochemists, and chemists.[4,12,112,143—146] Furoxanes are considered as prodrugs with a biological activity realized through the sGC—cGMP sequence.[142] The first stage of furoxane degradation is the attack by the thiolate anion on positions 3 and/or 5, resulting in ring dearomatization and ensuring the possibility of its opening followed by release of nitric oxide.

The thiol-dependent release of NO from furoxanes is presented in Scheme 58.[144]

Furoxanes are thermally stable and acid-resistant compounds; they react with electrophiles with difficulty. They are less stable with respect to bases or nucleophilic reagents and, as can be seen from the presented Scheme, decompose under the action of thiolate anions. Compared with other nitric oxide donors, furoxanes possess rather favorable pharmacological properties: they are slowly transformed and exert a long-term action.

The pharmacological effects characteristic of furoxanes were noted for 3-carbamoyl-4-hydroxymethyl derivative (CAS 1609).[147]



CAS 1609

The arguments confirming that this compound is a generator of nitric oxide are as follows:

(1) the effects due to this compound are inhibited by oxyhemoglobin;

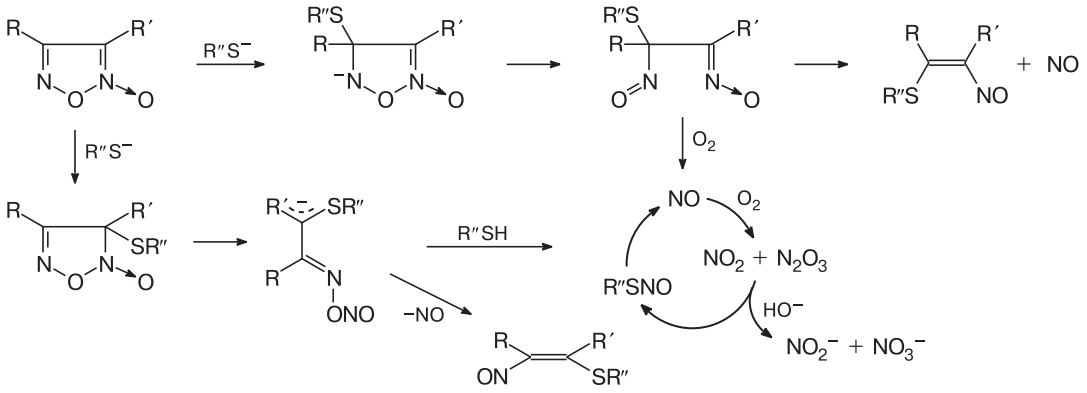
(2) administration of this compound is followed by a substantial increase in the cGMP content in organs and tissues;

(3) the use of this derivative entails a substantial decrease in blood pressure;

(4) apparent antianginal action.

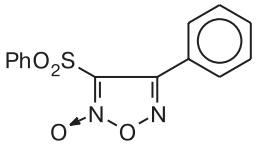
An important distinctive feature of furoxane action is the absence of tolerance. A specific feature of the pharmacological action of the orally active CAS 1609 is the

Scheme 58



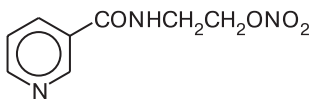
fact that this compound can be used to prevent coronary cardiac decompensation.

Yet another furoxane compound CHF 2206 eliminates the vasospasms caused by noradrenaline;[148] its activity in this respect is 4 times as high as that of nitroglycerin.[149] In addition, CHF 2206 inhibits platelet aggregation. A compound with a similar structure[149] containing an ethoxy group in position 4 instead of the phenyl group exhibits similar properties and does not give cross-tolerance with nitroglycerin.



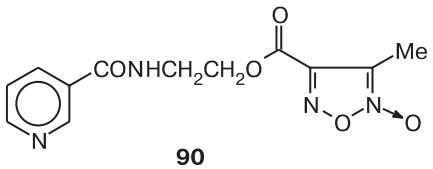
CHF 2206

The properties of another NO donor, nicorandil, which is actually a "hybrid" molecule combining the nitrate and nicotinamide fragments, have been studied.[149]

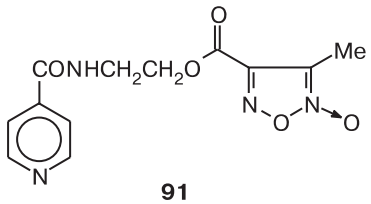


Nicorandil

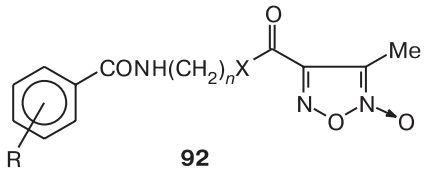
Nicorandil is known as an antianginal drug that combines the properties of an agent opening the potassium channels with the properties of a nitric oxide donor; this provides blood vessel relaxation by two mechanisms, one involving an increase in the potassium membrane conductivity due to opening of the ATP-dependent K^+ channels leading to membrane hyperpolarization, and the other involving NO release, leading to an increase in the cGMP level due to sGC stimulation. To continue studies along this line but with replacement of the nitrate NO donor by a furoxane one, a number of new compounds have been prepared. In compounds of this type,



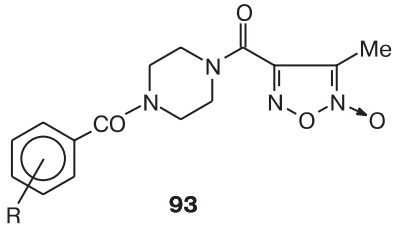
90



91

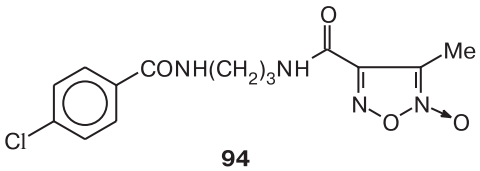


92

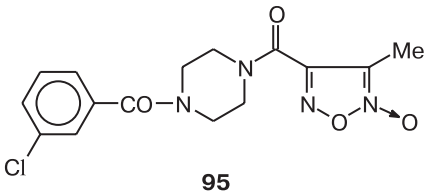


93

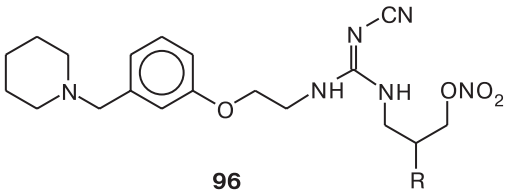
X = O, NH



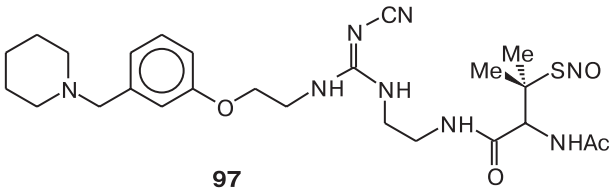
94



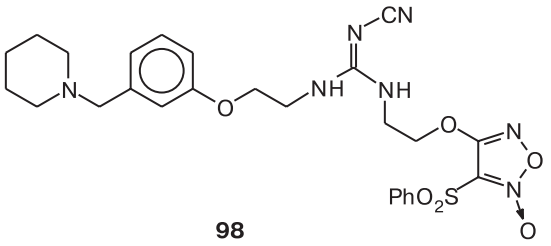
95



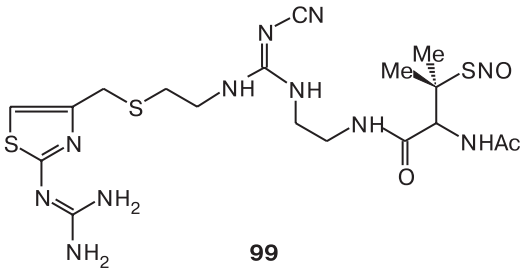
96



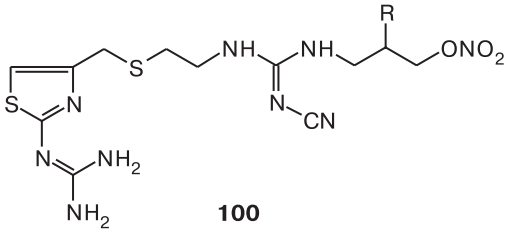
97



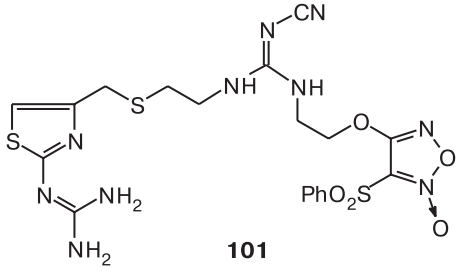
98



99



100



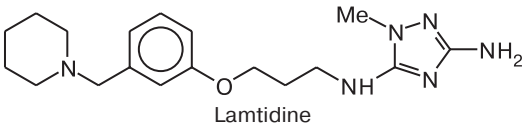
101

as in nicorandil, nicotinamide (and isonicotinamide) residues were chosen as the key fragments, but, instead of the nitrate group, a substituted furoxane residue served as the terminal substituent (**90**, **91**). Apart from pyridine-containing compounds, their benzene analogs were prepared **92**, **93**.[149]

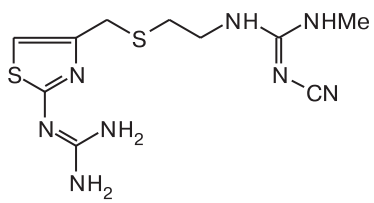
Each of the prepared compounds causes substantial vasorelaxation. Benzene derivatives proved to be the most active **94**, **95**.[149]

A compound containing a piperazine ring exhibited a long-term and pronounced hypotensive effect.

New gastroprotectors containing fragments capable of liberating nitric oxide, in addition to the groups traditional for these substances have been synthesized.[150] The purpose of this work was to combine the antisecretory activity, typical of H_2-blockers, with the gastroprotectory action of NO donors. Lamtidine and tiotidine were chosen as the basic molecules among blockers of histamine receptors (H_2-receptors)



Lamtidine

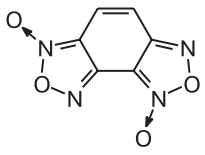


Tiotidine

The nitrate, thionitrosyl, and furoxane fragments were taken as the NO-donor fragments. The corresponding lamtidine (**96—98**) and tiotidine (**99—101**) derivatives were synthesized.

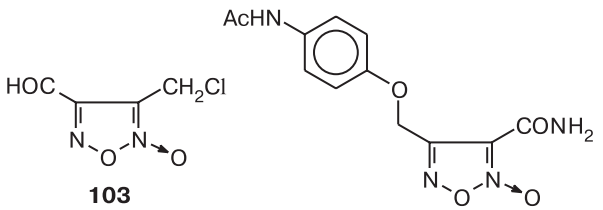
All the "hybrid" compounds thus obtained exhibited antisecretory and gastroprotectory activities, the lamtidine derivatives being more active. The best results were found for the "hybrids" containing a furoxane fragment. In the researchers´ opinion,[150,151] these compounds can serve as prototypes for a new class of drugs in the search for agents to control gastric hypersecretion.

It has been shown recently that the antihypertensive action of benzodifuroxane **102** [145] is due to the generation of nitric oxide, activation of soluble guanylate cyclase (sGC), and accumulation of cGMP. It was also found that compound **102** is a highly efficient NO-dependent inhibitor of platelet aggregation.



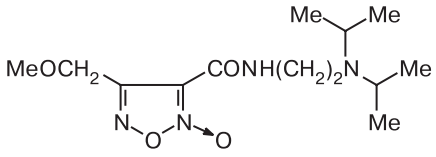
102

A number of studies have been devoted to the synthesis and pharmacological study of furoxane derivatives, which differ markedly in chemical structure (**103—105**) but exhibit similar biological effects (antianginal action and effects typical of nitric oxide donors useful for treatment of sexual dysfunction in men).[152]



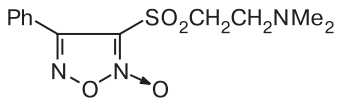
103

104



105

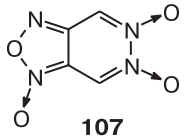
A water-soluble compound **106** was also synthesized and found to spontaneously release nitric oxide over a period of more than 24 h.[153]



106

In conclusion, we would like to note an interesting bicyclic compound, oxadiazolo[3,4-*d*]pyridazine tris-*N*-oxide (**107**).

It was shown[154] that this compound has a vasodilatory action; its effects include sGC activation.



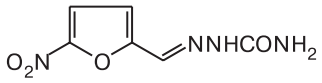
107

4.12. Known drugs capable of generating nitric oxide under hydrolytic, oxidative, or reductive conditions

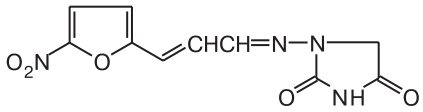
A number of well-known drugs widely used in medical practice, for example, nitrates (first of all, nitroglycerin and a sydnonimine derivative, molsidomine) are actually prodrugs whose biological action is due to their ability to release nitric oxide in the organism. However, these drugs had been discovered back before breaking of the "nitric oxide age", *i.e.*, before the role of NO in the functioning of living organism was discovered. Currently, it appears essential to return to the mechanism of action of those drugs that resemble in structure the substances capable of nitric oxide generation and to evaluate the contribution of this phenomenon to the biological action of these drugs.

The first group of preparations include well-known antimicrobial agents of the nitrofuran series. Nitrofurans are efficient with respect to both gram-positive and gram-negative bacteria. Since nitrofurans are known to undergo ring opening under the action of various nucleophiles, it was suggested that dearomatization of nitrofuran derivatives may take place under reductive conditions, which would give rise to compounds able to release nitric oxide.[155]

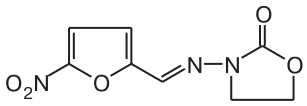
The behavior of some drugs of the nitrofuran series, *e.g.*, furacillin, furagin, furazolidone, under reductive conditions has been studied.[155]



Furacillin



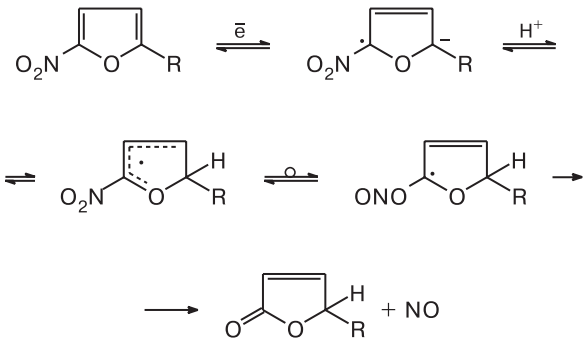
Furagin



Furazolidone

Heating of solutions of these compounds with potassium ferrocyanide at 60 °C and pH 5 gives rise to nitroprusside anion, which is indicative of the formation of nitric oxide under these conditions. The reduction of the nitrofuran derivatives with ascorbic acid (pH 6.5) also produces NO (reaction with potassium ferrocyanide). The radical anions resulting from the reduction of 5-nitrofurans can undergo the nitro→nitrite rearrangement.[156] With this in mind, the following mechanism for the formation of nitric oxide upon decomposition of drugs of the nitrofuran series was proposed[155] (Scheme 59).

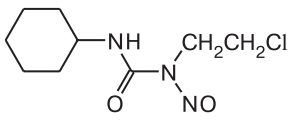
Scheme 59



As shown above, many effects of NO are related to the formation of peroxynitrite (see Sections 4.2, 4.10). The antimicrobial action of this species is due to inhibition of the electron transport chain in mitochondria, which violates cell respiration of microbes. In principle, it cannot be ruled out that the ability to generate nitric

oxide is one of the reasons for the high antimicrobial activities of these drugs.

The antitumor drug lomustine, which is an *N*-nitrosourea derivative, is also of interest in this respect.

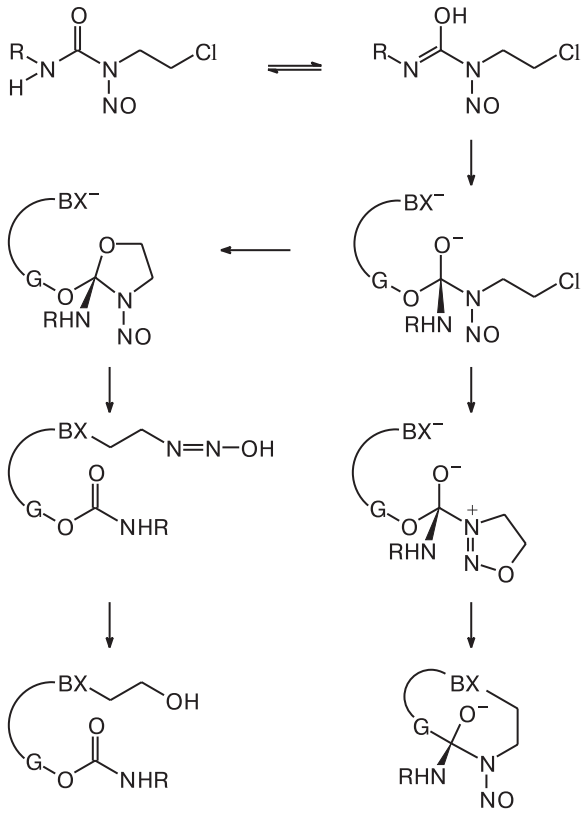


Lomustine

The presence of *N*-nitroso group in this and in other related compounds decreases the bond energy between the nitrosated nitrogen atom and the carbon atom of the neighboring carbonyl group; labilization of this bond is sufficient for bond cleavage even under physiological conditions to give electrophilic species, which can react with sensitive cell components.

At present, it is accepted that the key target of lomustine is DNA, its specific injuries being related to alkylation of bases and phosphate residues. The main route of chemical interaction of lomustine or other *N*-nitroso ureas with DNA is represented as alkylation of 6-hydroxy group of guanine[157] (Scheme 60).

Scheme 60

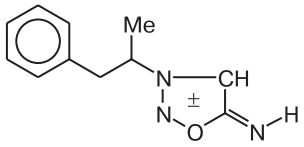


G—O is the guanidine residue, BX are DNA fragments.

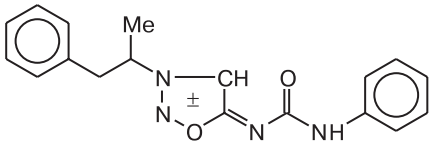
The possibility of formation of nitric oxide upon biotransformation, which may also be responsible for the antitumor activity of these compounds, has been studied using lomistine as an example.[158] The extensive literature on the antitumor activity of nitrosourea derivatives virtually has not considered this possibility.

It was found that hydrolysis of lomustine in solutions yields nitrite anion, which can be detected by polarography; the reaction with potassium ferrocyanide affords nitroprusside ion, which attests to NO liberation as a result of hydrolytic transformations.

The pharmacological activity of the well-known cardiovascular drug, molsidomine, is due to the release of nitric oxide during its biotransformation (see Section 4.11.1). These data were taken into account when considering the pathways of probable biotransformation of psychotropic agents of the mesoionic structure, namely, sydnophen and sydnocarb.[159] The structures of both drugs incorporate a β-phenylalkylamino group.



Sydnophen

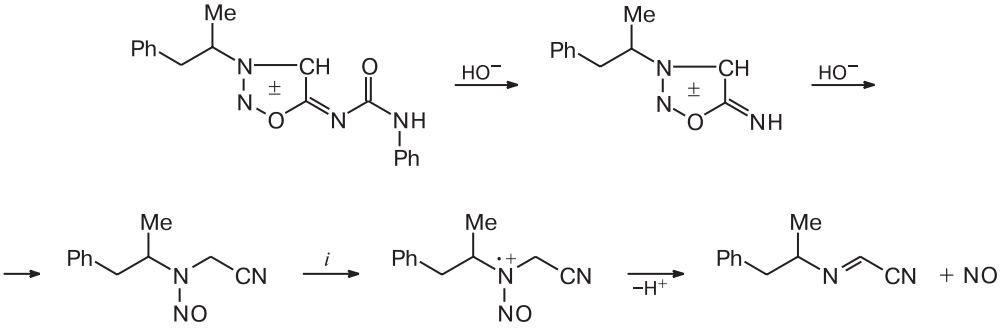


Sydnocarb

The mechanisms of action of these compounds were studied.[41,81] New data concerning the biological activity of sydnophen and sydnocarb were reported.[159] Sydnophen is known to inhibit monoamine oxidase, which accounts for its antidepressant action. The effective psychostimulant sydnocarb is an indirect sympathomimetic. The similarity of the structures of these sydnonimine derivatives to the structure of the popular antihypertensive drug molsidomine, which is a nitric oxide donor, suggests that both sydnophen and sydnocarb can generate NO in the organism. By analogy with molsidomine, the pattern of NO formation can be represented in the following way (Scheme 61).

A polarographic study of the hydrolysis of sydnocarb and sydnophen at pH 8.2 showed that this process yields *N*-nitroso-*N*-(α-benzyl)ethylaminoacetonitrile, which is oxidized by potassium ferricyanide to give nitric oxide. The oxidation step is an obligatory condition for nitric oxide release; indeed, no nitric oxide is formed under anaerobic conditions. Note also that in the case of sydnocarb, hydrolysis of the phenylcarbamoyl fragment is necessary in the first stage. Thus, these experiments

Scheme 61

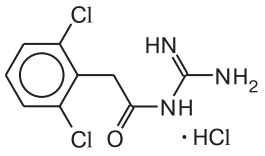


i. Oxidation.

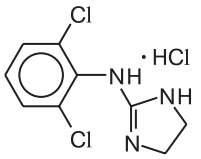
simulate the sequence of transformations of a known NO donor, molsidomine, and, therefore, nitric oxide release is likely to occur in the organism on treatment with these drugs. However, no proof of the importance of this process for the pharmacological activity of mesoionic agents has yet been obtained. Solution of this problem requires special biochemical and pharmacological research.

Yet another new route in the search for nitric oxide donors among known drugs is based on investigation of substances containing a guanidine fragment. This approach appears quite natural, because the guanidine NH group of L-arginine is responsible for the nitric oxide formation under physiological conditions (see Section 2).

In the first stage, guanfacine and clonidine were chosen as the subjects of investigation.



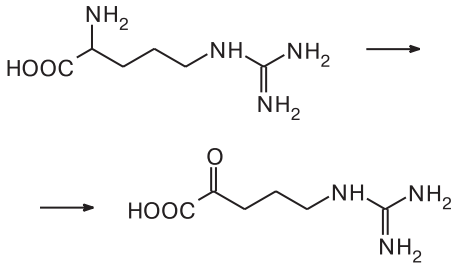
Guanfacine



Clonidine (Clophelin)

These drugs are α_2-adrenergic receptor stimulants; they reduce the release of the noradrenaline mediator from the nerve endings, which finally results in a hypotensive effect. Both compounds can act as substrates for NO synthases (see Section 2). It was shown[160] that on oxidation they eliminate nitric oxide, the NO-donor activity of guanfacine being higher and that of clonidine, which contains a cyclic guanidine fragment, being somewhat lower than that of L-arginine (the main source of NO in the organism). It is pertinent to mention here an interesting finding,[161] namely, the fact that the pathway of chemical oxidation of L-arginine by potassium ferricyanide in an alkaline medium differs from that of oxidation by NO-synthases, in particular, it gives an oxo acid rather than citrulline and does not give NO (Scheme 62).

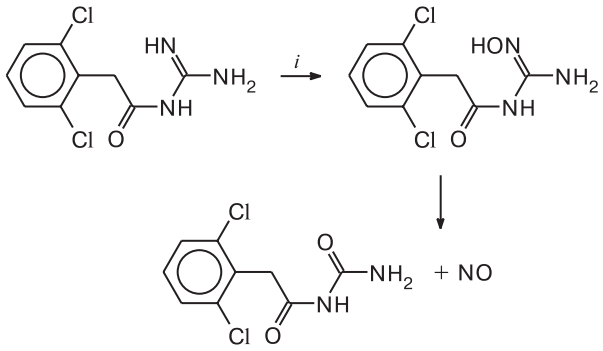
Scheme 62



Under conditions used for the oxidation,[160] all the compounds studied including arginine give nitric oxide.

It was also shown that guanfacine and clonidine induce drug concentration-dependent activation of sGC. It was suggested that oxidation of guanfacine, containing an "open" guanidine fragment, follows the same mechanism as L-arginine oxidation (Scheme 63).

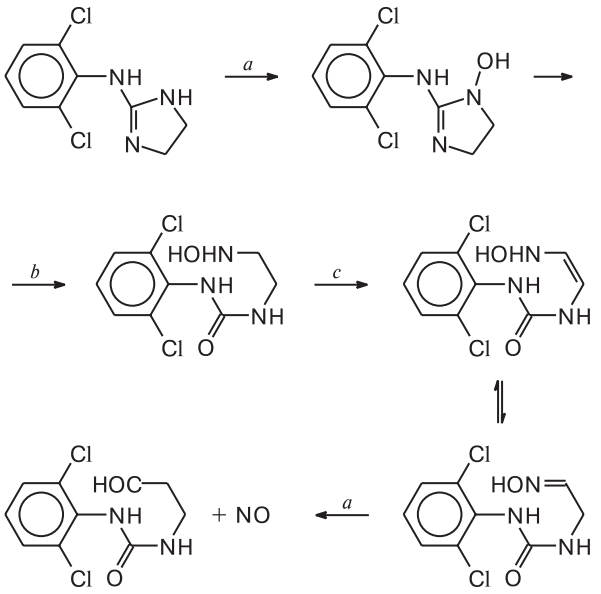
Scheme 63



i. Oxidation.

In the case of clonidine, the presence of cyclic dihydroimidazole system is an appreciable complication, and the hypothetical pathway of oxidation of this drug is as follows (Scheme 64).

Scheme 64



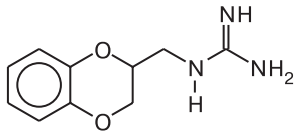
a. Oxidation. *b*. Hydrolysis. *c*. Dehydrogenation.

The key steps of this sequence are dehydrogenation and oxidation of the resulting oxime (along the pathway known for oximes, see Section 4.6). An attempt was made to accelerate dehydrogenation by the addition of NAD^+; the activation sGC under the action of clonidine was shown to be enhanced in the presence of this coenzyme. This validates the above scheme.

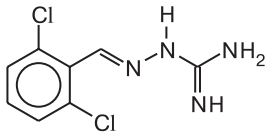
The data of Table 5 show that the activation of sGC by the guanidine derivatives under study is significant. For guanfacine, it was additionally found that the level of sGC activation decreases appreciably in the presence of NOS inhibitor, *N*-methylarginine.

Although the ability of clonidine and guanfacine to release NO on oxidation and to activate sGC was established reliably, this cannot be regarded as a ground for revision of the current views on the mechanism of action of these medical drugs. Nevertheless, the new data deserve attention of pharmacologists and biochemists as regards further research into the roots of the pharmacological action of these preparations.

The antihypertensive drugs, guanoxan and guanabenz are examples of known drugs whose molecules incorporate a guanidine fragment.



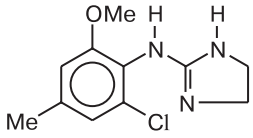
Guanoxan



Guanabenz

Treatment of guanfacine, guanoxan, and guanabenz with sodium hypochlorite (10^{-3} mol L^{-1}) at 60 °C for 30 min at pH 9.2—11 was shown[159] to give nitric oxide in 2—3%, 4—6%, and 4—6%, respectively. Both guanoxan and guanabenz activate sGC.

One more substance that has attracted attention as a probable nitric oxide donor is moxonidine, which, like clonidine, contains a cyclic guanidine fragment.



Moxonidine

This long-acting antihypertensive drug is an imidazoline receptor agonist. Oxidation of moxonidine with sodium hypochlorite under the conditions indicated above affords NO in 2—3% yield. Moxonidine stimulates sGC. As for clophelin, the addition of NAD^+ increases in some cases the level of sGC activation, although this is not clearly defined and shows itself only at some concentrations (Table 6).

To conclude this section, we shall consider the known antiprotozoal drugs, metronidazole and nitazole.

Metronidazole possesses a broad chemotherapeutical spectrum of action with respect to anaerobic protozoa

Table 5. Activation of sGC (*a*) in the presence of clonidine (clophelin) and guanfacine*

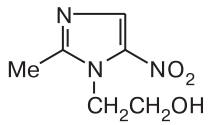
Drug	*a*/act.u. at *C***/mol L^{-1}			
	10^{-7}	10^{-6}	10^{-5}	10^{-4}
Clonidine (clophelin)	—	1.0±0.1	0.9±0.1	1.3±0.1
Clonidine + NAD^+ (10^{-7} mol L^{-1})	—	—	1.1±0.1	—
Clonidine + NAD^+ (10^{-6} mol L^{-1})	—	—	1.7±0.1	—
Guanfacine	1.7±0.1	1.8±0.1	1.4±0.1	1.2±0.1
Guanfacine + NAD^+ (10^{-6} mol L^{-1})	1.3±0.1	1.4±0.1	—	—

* The initial activity was taken to be unity.
** Drug concentration.

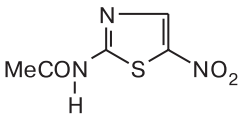
Table 6. Activation of sGC (*a*) in the presence of moxonidine

Initial activity	*a*/act.u. at *C**/mol L^{-1}		
	10^{-6}	10^{-5}	10^{-4}
0.89±0.10	0.98±0.06	0.16±0.11	0.89±0.07
1.51±0.11 (NAD^+, 10^{-7} mol L^{-1})	2.31±0.18	1.78±0.12	—
1.53±0.13 (NAD^+, 10^{-6} mol L^{-1})	1.60±0.09	1.82±0.13	—

* Moxonidine concentration.



Metronidazole



Nitazole

and to anaerobic gram-negative and some gram-positive bacteria. Using electrochemical techniques and ESR, it was shown that metronidazole is readily converted into the product of single-electron reduction of the nitro group (radical anion), capable of violating structures of DNA, RNA, and other vitally important cell macromolecules, and it is this radical anion that seems to be responsible for the biological activity of the drug.[162] The question of the mechanism of action of metronidazole cannot be regarded as ultimately answered. For example, there is an opinion that the hydroxylamine derivative resulting from its reduction is the active form of the drug.[163] In this respect, one should pay attention to the possible elimination of the nitro group upon the reduction of metronidazole.[164—166] It is known that electrolysis of metronidazole gives nitrite ions in high yield;[164] the reduction of the drug in solution in the presence of thiols and two-charged iron ions affords complexes incorporating iron ions, thio derivatives, and nitric oxide.[165] Primary attention should be devoted to the fact that *N*-substituted 5-nitro-2-styrylimidazoles undergo destruction in strongly alkaline media (pH > 11) with opening of the imidazole ring and elimination of the nitro group as nitrite anion.[167] These data provided the basis for the conclusion[168] that the release of nitric oxide is a probable route in the metronidazole biotransformation and that this circumstance may play an important role in the mechanism of its biological action.

Alkaline hydrolysis of metronidazole was found to yield nitric oxide. The rate constant for the hydrolysis in a 0.01 *M* solution of NaOH at 80 °C amounts to $1.6 \cdot 10^{-3}$ min^{-1}. In 0.1 *M* alkali, elimination of the nitro group from metronidazole proceeds almost quantitatively over a period of 30 min.

The release of nitric oxide upon degradation of metronidazole is confirmed by the fact that this drug activates sGC. The NO-donor properties found for metronidazole stimulated us[168] to evaluate the influence of this drug on the hemodynamic parameters, such as the cardiac frequency and the systemic arterial blood pressure in animals, and to compare the results with those for a typical NO donor, isosorbide mononitrate. It was found that the effects of metronidazole and isosorbide mononitrate are rather similar.

The pathway to nitric oxide can be represented by Scheme 65.

However, it cannot be ruled out that in this case, too, the nitro→nitrite rearrangement can proceed in a certain stage (see Section 4.12).

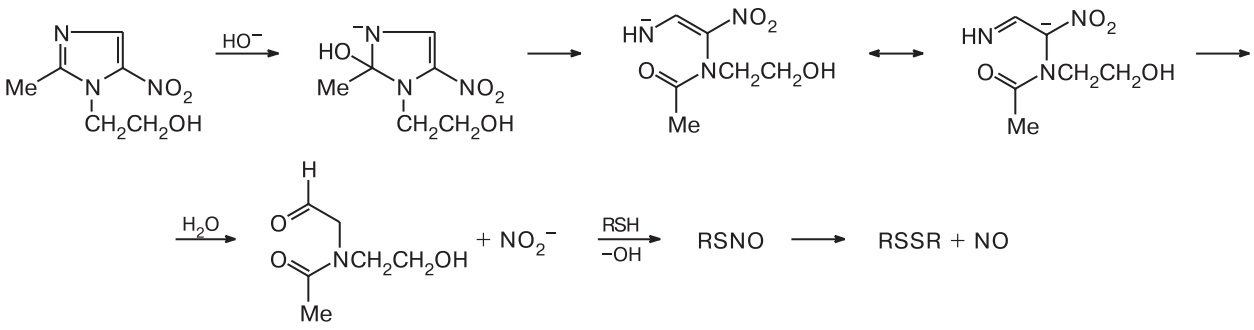
In order to consider the pathway to nitric oxide upon reduction, one requires additional data.

Analogous information concerning nitazole, which also releases NO under the above-indicated conditions and activates sGC, is avbailable.

It can be seen from the presented data that various medications have been established reliably to release nitric oxide *in vitro* and, in many cases, also *in vivo*. For some of these drugs (nitrates, *S*-nitrosothiols, sodium nitroprusside, molsidomine), it is obvious that the capability of forming NO is responsible for the principal action. For some other drugs, nitric oxide formation is a side effect and elucidation of its role in their biological action still requires further research.

At the end of this section, let us summarize the routes of nitric oxide formation when some types of drugs are used. The release of nitric oxide can follow either chemical or enzymatic mechanism.[169] Diazenium-1,2-diolates tend to undergo chemical degradation, while enzymatic degradation is typical of nitrates. Both ways of NO generation are essential for syndonimines, sodium nitroprusside, nitrite, and *S*-nitrosothiols. The chemical release implies reactions of NO donors with thiols and other reducing agents such as ascorbic acid.[169] The enzymatic routes have not been fully characterized yet. It is known, however, that the release of NO from nitroglyc-

Scheme 65



erin is catalyzed by glutathione-S-transferase,[169] and this process involves also cytochrome P-450 and microsomal enzymes of the smooth-muscle cells of vessels.

5. Nitric oxide and some pathological processes occurring in the living organism

The first point that should be noted in this Section is the influence of nitric oxide on apoptosis. Apoptosis is a fundamental process in the vital activity of multicellular organisms.[170,171] The term apopotosis is used to denote an active process of cell destruction, characterized by cell compression, chromatin aggregation with extensive genome fragmentation, and nucleus destruction. This process results in the removal of cells that have lost their functions and have become potentially hazardous for the organism (mutant cells, virus infected cells, and so on). Unlike the cell death caused by various damaging agents of the biological, physical, and chemical natures (necrosis), apoptosis is a physiological cell "suicide" (this phenomenon was described back in 1951[172] but the concept was developed only in the 1980s—1990s), which is an adaptive mechanism that maintains the dynamic equilibrium between the cell proliferation and elimination. Apoptosis comprises active energy-dependent processes resulting in higher concentrations of calcium ions and cAMP and in activation of various enzymatic systems. All this suggests that nitric oxide might participate in these processes. Unfortunately, data concerning this participation still remain quite scarce and here we refer (except for a review[171]) only to one study.[173] It was shown in this study that exogenous nitric oxide is an apoptosis mediator in the rheumatoid arthritis, whereas inhibitors of inducible NO synthase (*i.e.*, compounds preventing nitric oxide formation in inflammatory processes) inhibit apoptosis.

Atherosclerosis and hypercholesterolemia deteriorate the NO-dependent vessel tone regulation. Nitric oxide formed in the endothelium is a potent endogenous vasodilatory agent, and under normal physiological conditions a strictly defined NO distribution is maintained in tissues, depending on the rates of the laminar, turbulent, and pulsating blood flows. Inhibition of the synthesis of nitric oxide is a prerequisite for the appearance and progressing of atherosclerosis.[174] In this connection, mention should be made of a number of pathophysiological factors leading to anomalies in the formation and functions of NO in the organism. These include injury of receptor membranes in arteries where nitric oxide is generated, a decrease in L-arginine concentration and in the activity of NO synthase, a decrease in the possibility of nitric oxide release from the arteries damaged by atherosclerosis. The adverse effects also include the obstacles to nitric oxide diffusion to the smooth-muscle cells, a decrease in the sensitivity to its vasodilatory action, the enhancement of nitric oxide degradation caused by more active generation of free radicals, and violation of the mechanisms of NO interaction with sGC accompanied by a decrease in the cGMP formation. The proposed strategy for the therapy of atherosclerosis implies combined use of L-arginine and antioxidants for maintaining and/or restoring the NO regulation in the arteries.[174]

The formation of nitric oxide in the organism is accompanied by its reaction with superoxide radical anion giving rise to peroxynitrite anion responsible for the nitration of tyrosine residues in proteins,[132,175,176] for NO cytotoxicity, for tissue injury, and for development of atherosclerosis. Low doses of nitric oxide and peroxynitrite promote antiatherosclerotic changes, but in atherosclerosis, their toxic effect can develop due to peroxynitrite accumulation in atherosclerosis plaques.[176]

A study of pentaerythritol tetranitrate as a nitric oxide donor[34] (see Section 4.2), showed that nitric oxide formed from exogenous NO donors exhibits antisclerotic and vasoprotector effects.

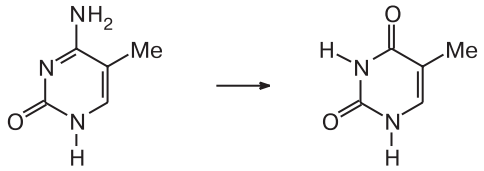
Nitric oxide also influences angiogenesis.* Angiogenesis is initiated by vasodilation and is also promoted by the vascular endothelial growth factor (VEGF), which increases their permeability. Nitric oxide promotes angiogenesis[175] and, for instance, its blocking in tumors is attained by inhibition of iNOS. The nitric oxide production may be responsible for both acceleration of tumor growth and for tumor size reduction due to apoptosis (see above). For example, nitric oxide formed from sodium nitroprusside is known to markedly reduce the growth of pancreatic tumor cells. The relationship between nitric oxide and angiogenesis was confirmed for the cardiac infarction.[175] Nitric oxide donors and growth factors induce angiogenesis. Thus, in the case of cardiac infarction, nitric oxide plays a positive role because it induces a new vascular growth. Therefore, it is considered[175] that NO may be recommended for the therapy of diseases caused by disorder of the myocardium blood supply.

The existing data on the possibility of using nitric oxide generators against neoplastic processes are intricate and contradictory. These problems have been considered in more detail in reviews.[177,178] It is believed[11] that nitric oxide may be cytotoxic with respect to tumor cells but it can be also act as a carcinogen, for example, by nitrosating endogenous secondary amines to give carcinogenic *N*-nitroso derivatives. In addition, NO can cause mutagenesis by transforming 5-methylcytosine into thymine (Scheme 66).

No nitric oxide donors that could be regarded as effective means for cancer treatment have been found yet. However, the use of compounds of this type in the integrated approach to cancer therapy, for example, to

* Angiogenesis is the formation of blood vessels, which plays an important role in tumor development by promoting their growth.

Scheme 66



5-Methylcytosine Thymine

optimize the action of chemotherapeutic agents or together with radiotherapy can increase the efficiency of treatment. It is beyond doubt that active research aimed at selecting new NO generators (or NOS inhibitors) is promising and worthwhile.

Yet another important property of nitric oxide that is currently under study is the influence on the origin of migraine.[179] Although the causes of migraine are not entirely clear, the available data indicate that administration of nitroglycerin often leads to migraine. Presumably, this is due to excessive nitric oxide release. It cannot be ruled out[179] that migraine is caused by physiological hypersensitization of an organism to nitrogen oxide. It has been suggested that the release of NO from the blood vessels surrounding the vascular nerve endings or from brain tissues constitutes the molecular mechanism of migraine origination.[179] This hypothesis is supported by the fact that the introduction of NOS inhibitors is efficient for patients with acute migraine. The use of selective eNOS, nNOS, and iNOS inhibitors can prove rather important for the understanding of the molecular mechanism and for treatment of this disease.

6. Problems of NO investigation *in vitro* and *in vivo*

The question of to what extent experiments with separate organs, *i.e.*, *in vitro* experiments, can be transferred to the whole organism is a general biological problem, and the whole set of research concerning NO-producing substances needs, of course, to be correctly evaluated if the data have been obtained at the cellular level. Indeed, the very first question arising if the experiments have been carried out with isolated organs is whether the cells under study are able to activate the chosen class of nitric oxide donors if the compounds of this class require enzymatic biotransformation. A commonly cited example is the fact that a number of compounds exhibit a fairly low level of antiaggregatory activity if experiments are carried out with washed platelets, which do not contain enzymes needed to metabolize these drugs.[181]

The *in vivo* problems are also quite diverse. One of the most important problems is, perhaps, the extremely limited tissue specificity of most of NO donors. Due to the extremely large variety of functions played by nitric oxide in a living organism, it is exceptionally difficult to find a compound that would generate NO and, simultaneously, have only one physiological function. Therefore, when such substances are used, nitric oxide is often released not at the site where it is required. One avenue along which the chemistry of nitric oxide donors is currently developing and which can be clearly traced while thoroughly examining the data reported in this review is the broad variation of lipophilic (or hydrophilic) substituents (around an NO-generating site) in order to ensure the targeted delivery of compounds into tissues and to decrease or limit the adverse systemic effects. The lipophilicity of NO donors is a very important feature. Where it is low, they cannot penetrate biological barriers, especially the blood—brain barrier and do not modulate NO-dependent processes in the central nervous system. In the case of high lipophilicity, the positive processes at the periphery overlap with adverse effects in the central nervous system.

Here, one should pay attention to the fact that the vasodilatory properties of NO donors restrict the possibility of using them against pathologies that do not require decrease in blood pressure. Presumably,[181] nitrates are preferred for the coronary vessel dilation because they are not bioactivated in resistant arteries and do not, unlike other NO donors, cause the coronary blood outflow.

7. Conclusion

Now we summarize the discussion of the most important nitric oxide donors and their key characteristics.

1. Organic nitrates (see Section 4.2). The biological effects of these compounds are mainly due to their ability to release nitric oxide, their bioactivation proceeding due to the reaction with thiols. It is not clear yet whether thiols serve as enzyme co-factors or merely as reducing agents in each particular case.[181] It was stated[11] that nitric oxide can be released either enzymatically or nonenzymatically; however, thiols are involved in both pathways. In the case of nonenzymatic activation of organic nitrates, thiols are converted into disulfides and nitrite anions are formed. The latter species are transformed into thionitrates and then into nitric oxide. All thiols are capable of decomposing nitrates but not all of them produce NO. Cysteine and *N*-acetylcysteine, unlike glutathione, enhance the formation of nitric oxide. Presumably, the NADPH-dependent cytochrome P-450 and some glutathione-S-transferase isoenzymes are used in enzymatic bioactivation. The introduction of L-cysteine substantially enhances the nitroglycerin-induced dilation of small coronary vessels. It was shown[181] that when nitrates are used, nitronium cation is generated together with nitric oxide.

2. *S*-Nitrosothiols (see Section 4.5). No direct evidence for enzymatic decomposition of *S*-nitrosothiols

has been reported so far. Homolytic cleavage of these compounds in the presence of metal ions, for example, Cu^+ and Fe^{2+}, affords nitric oxide and thiyl radicals, the latter being probably responsible for the mutagenic action of these compounds. The heterolysis of *S*-nitrosothiols affords NO^+ and NO^-. The low-molecular-mass *S*-nitrosothiols are less stable than high-molecular-mass compounds. The SNO groups in proteins may serve for depositing nitric oxide followed by its transport to the appropriate targets.[181] *S*-Nitrosothiols enter into *trans*-nitrosation. This can be accompanied by reversible protection of the thiol groups of proteins. Thus, *S*-nitrosothiols can exhibit pharmacological effects caused by nitrosation of cell proteins rather than by release of nitric oxide.

3. Guanidines (see Sections 4.1 and 4.12). The transformation of both endogenous and exogenous guanidine derivatives with evolution of nitric oxide takes place under oxidative conditions and clearly resembles the main prototype, *i.e.*, NO synthesis under physiological conditions upon the action of NOS on L-arginine.

It has been shown in recent years that a number of antihypertensive agents containing a guanidine fragment can act as nitric oxide donors. The existing views on the mechanisms of their action require more detailed elaboration.

4. Sydnonimines (see Section 4.11.1). The action of some drugs of this type is undoubtedly caused by the release of nitric oxide. Other reasons for their activity also exist. Under aerobic conditions, SIN-1 yields nitric oxide together with superoxide radical anion, which gives rise to peroxynitrite, responsible for injury effects. Oxidation of SIN-1 under anaerobic conditions affords neither superoxide radical anion nor peroxynitrite.

5. Diazenium-1,2-diolates (see Section 4.9). The activity of diazenium-1,2-diolates correlates with the rates of nitric oxide release. The degradation of diazenium-1,2-diolates gives NO, NO^-, and NO^+.[181] The conversions of compounds of this types, which are prodrugs, requires appropriate hydrolases.

6. *C*-Nitro derivatives (see Sections 4.10 and 4.12). The manifestation of NO-donor properties by compounds of this type requires either powerful activation of nitro groups by electron-withdrawing substituents, or the ability of nitro compounds to be rearranged into the corresponding *O*-nitroso derivatives, which can easily eliminate nitric oxide, or ring (heterocycle) dearomatization to give acyclic systems.

7. A promising avenue in the search for new drugs is the design of "hybrid" molecules, *i.e.*, molecules incorporating a fragment capable of performing NO-donor function in addition to a moiety representing the structural basis of a known drug.

Some aspects of this problem have been considered in a recent review.[182] Numerous publications show that nitric oxide acts as a multifunctional gastroptective mediator affecting some aspects of the gastrointestinal tract physiology including mucus and bicarbonate secretion, blood flow in the gastrointestinal tract walls, and antiinflammatory cell responses. The data indicating that nitric oxide donors can efficiently suppress injuries of the gastric mucous membrane and accelerate curing of the gastrointestinal tract make these compounds important for the elimination of the gastropathy induced by nonsteroidal antiinflammatory drugs (NSAID) when taken chronically.[183—186] The use of "hybrid" compounds, unlike the NSAID themselves, does not cause ulcerogenic effect and, moreover, accelerates healing of the existing ulcer injuries.

It should be emphasized that the pattern of involvement of nitric oxide in various physiological processes cannot be complete unless one considers not only NO donors but also NO-synthase inhibitors, because the NO balance is accurately adjusted only in a young and healthy organism. A whole series of pathological conditions are largely related to the deficiency of nitric oxide in the organism. In this connection, it is clear that the search for various compounds able to serve as nitric oxide donors in the organism, *i.e.*, for xenobiotics whose transformation may give NO, is currently among the most actively progressing lines of investigations.

However, all this does not refute the fact that the deficiency of nitric oxide is not the only cause of the pathologies related to this regulatory agent. Excessive release of nitric oxide also induces a series of pathological conditions, in particular, cardiovascular diseases, cerebropathy, toxicological effects such as extremal hypotension (sharp decrease in the blood pressure), cardiovascular collapse, cell damages, or septic shock.

For acquaintance with the problem of NOS inhibitors, we refer to some reviews published in recent years.[3,4,187,188] In the near future, we intend to supplement the material of this review by a survey of the published data on the chemistry and biology of NO-synthase inhibitors.

References

1. S. Moncada, R. M. S. Palmer, and E. A. Higgs, *Pharmacol. Rev.*, 1991, **43**, 109.
2. M. A. Marletta, *J. Med. Chem.*, 1994, **37**, 1899.
3. O. W. Griffith and D. J. Stuehr, *Annu. Rev. Physiol.*, 1995, **57**, 707.
4. V. G. Granik, S. Yu. Ryabova, and N. B. Grigor´ev, *Usp. Khim.*, 1997, **66**, 792 [*Russ. Chem. Rev.*, 1997, **66** (Engl. Transl.)].
5. A. F. Vanin, *Biokhimiya*, 1998, **63**, 924 [*Biochemistry (Moscow)*, 1998, **63** (Engl. Transl.)].
6. I. S. Severina, *Biokhimiya*, 1998, **63**, 939 [*Biochemistry (Moscow)*, 1998, **63** (Engl. Transl.)].

7. E. B. Men´shikova, N. K. Zenkov, and V. P. Reutov, *Biokhimiya*, 2000, **65**, 485 [*Biochemistry (Moscow)*, 2000, **65** (Engl. Transl.)].
8. *Nitric Oxide. Basic Research and Clinical Application*, Eds. R. J. Gryglewsky and P. Minuz, IOS Press, Amsterdam—Berlin—Oxford—Tokyo—Washington, DC, 2001.
9. D. Voet and J. G. Voet, *Biochemistry*, J. Wiley and Sons Inc., New York—Chichester—Brisbane—Toronto—Singapore, 1995, 2th ed., p. 1290.
10. F. Murad, *Neurotransmissions*, 1994, **10**, 1
11. H. Al-SaDoni and A. Ferro, *Clinical Science*, 2000, **98**, 507.
12. M. Feelish, *J. Cardiovascular. Pharm.*, 1991, **17** (Suppl 3), 25.
13. P. L. Feldman, O. W. Griffith, and D. J. Stuehr, *Chem. Eng. News*, 1993, **71**, 26.
14. M. A. Marletta, *J. Biol. Chem.*, 1993, **268**, 12231.
15. B. Clement, M.-H. Shultze-Mosgau, H. Wohlers, and F. Jung, in *Enzymology and Biochemistry*, Eds. M. Feelish, R. Busse, and S. Moncada, London, Portland Press Ltd, 1994, 15.
16. D. Monsuy, J. L. Boucher, and B. Clement, *Biochimie*, 1995, **77**, 661.
17. O. W. Griffits and D. J. Stuehr, *Annu. Rev. Physiol.*, 1995, **57**, 707.
18. N. E. Rogers and L. J. Ignarro, *Biochem. Biophys. Research Communs*, 1992, **189**, 242.
19. Yu. M. Petrenko, D. A. Shashurin, and V. Yu. Titov, *Eksperim. Klinich. Farmakologiya* (*Exp. Clinical Pharmacology*) 2001, **64**, 72 (in Russian).
20. J. Kling, *Modern Drug Discovery*, 1998, 31.
21. V. G. Granik, N. B. Grigoriev, L. H. Vinograd, I. S. Severina, M. D. Mashkovskyi, V. B. Nikitin, G. N. Engalycheva, M. A. Kalinkina, and O. G. Busigina, *Mendeleev Commun.*, 1996, **4**, 161.
22. V. I. Levina, D. A. Grigor´ev, and N. B. Grigor´ev, *Khim.-farm. zhurn.*, 1995, **29**, № 8, 56 [*Pharm. Chem. J.*, 1995, **29** (Engl. Transl.)].
23. B. Clement, J. L. Boucher, and D. Monsuy, *Biochem. Pharmacol.*, 1999, **58**, 439.
24. J. M. Fukuto, G. C. Wallace, R. Hszieh, and G. Chaudhuri, *Biochem. Pharmacol.*, 1992, **43**, 607.
25. V. A. Makarov, A. V. Danilov, V. I. Levina, N. B. Grigor´ev, M. Shmidke, A. Shtel´tsner, and V. G. Granik, *IX Ross. Natsional´nyi kongress "Chelovek i lekarstvo"* [*IX Russ. National Congress "Human and Drugs"*], *Abstrs.*, Folium, Moscow, 2002, 653 (in Russian).
26. S. Yu. Ryabova, Yu. I. Trofimkin, L. M. Alekseeva, I. F. Kerbnikova, G. Ya. Shvarts, and V. G. Granik, *Khim.-Farm. Zhurn.*, 1995, **29**, № 9, 22 [*Pharm. Chem. J.*, 1995, **29** (Engl. Transl.)].
27. S. Yu. Ryabova and V. G. Granik, *The XIVth Intern. Symp. on Medicinal Chemistry (Abstrs. of Reports)*, Maastricht, 1996, P-7.22.
28. M. A. Moro, V. M. Darley-Usmar, I. Lizasoain, Y. Su, R. G. Knowles, M. W. Radomski, and S. Moncada, *Brit. J. Pharmacol.*, 1995, **116**, 1999.
29. M. A. Moro, V. M. Darley-Usmar, D. A. Gudwin, N. G. Read, R. Zamora-Pino, M. Feelish, M. W. Radomski, and S. Moncada, *Proc. Natl. Acad. Sci. USA*, 1994, **91**, 6702.
30. R. Cereda, *Drug Future*, 1997, **22**, 242.
31. B. A. Sidorenko, D. V. Preobrazhenskii, and S. Yu. Zaikin, *Kardiologiya*, 1998, **38**, 84.
32. *Annual Drug Data Rep.*, Ed. J. R. Prous, 1994, **16**, 533.
33. *Annual Drug Data Rep.*, Ed. J. R. Prous, 1998, **20**, 768.
34. *Annual Drug Data Rep.*, Ed. J. R. Prous, 1997, **19**, 991.
35. A. Hacker, S. Muller, V. Meyer, and G. Koida, *Brit. J. Pharmacol.*, 2001, **132**, 1707.
36. S. Endres, A. Hacher, E. Noak, G. Koida, and J. Lehman, *Eur. J. Med. Chem.*, 1999, **34**, 895.
37. *Annual Drug Data Rep.*, Ed. J. R. Prous, 1997, **19**, 206.
38. *Annual Drug Data Rep.*, Ed. J. R. Prous, 1999, **21**, 891.
39. *Annual Drug Data Rep.*, Ed. J. R. Prous, 1999, **21**, 787.
40. L. Korzycka, H. Szmigielska, and E. Czarnecka, *Acta Poloniae Pharmaceutica — Drug Research*, 2000, **57**, 419.
41. V. G. Granik, *Lekarstva. Farmakologicheskii, biokhimicheskii i khimicheskii aspekty* [*Drugs. Pharmacological, Biochemical, and Chemical Aspects*], Vuzovskaya kniga, Moscow, 2001 (in Russian).
42. *Annual Drug Data Rep.*, Ed. J. R. Prous, 1997, **19**, 738.
43. J. L. Burgaud, F. Benedini, E. M. Robinson, and P. Del Soldato, *Drug Future*, 1999, **24**, 858.
44. N. B. Grigor´ev, M. A. Kalinkina, G. V. Chechekin, V. B. Nikitin, G. N. Engalycheva, N. N. Belushkina, V. I. Levina, I. L. Dalinger, M. D. Mashkovskii, S. A. Shevelev, V. A. Litosh, M. V. Veper, I. S. Severina, M. E. Kaminka, A. P. Arzamaztsev, and V. G. Granik, *Khim.-Farm. Zhurn.*, 1998, **32**, № 3, 15 [*Pharm. Chem. J.*, 1998, **32** (Engl. Transl.)].
45. B. Xuan, Y.-H. Zhou, R. Varma, and G. C. Y. Chiou, *J. Ocular Pharmacol. Therapeutics*, 1999, **15**, 135.
46. R. S. Varma, B. Xuan, and G. C. Y. Chiou, *J. Ocular Pharmacol. Therapeutics*, 2000, **16**, 571.
47. *Annual Drug Data Rep.*, Ed. J. R. Prous, 1997, **19**, 833.
48. *Annual Drug Data Rep.*, Ed. J. R. Prous, 1997, **19**, 834.
49. D. R. Arnelle and J. S. Stamler, *Arch. Biochem. Biophys.*, 1995, **318**, 279.
50. I. S. Severina, O. G. Bussygina, and N. B. Grigoriev, *Biochem. Intern.*, 1992, **26**, 695.
51. A. R. Butler, F. W. Flitney, and D. L. H. Williams, *Trends Pharmacol. Science*, 1995, **16**, 18.
52. I. L. Megson, I. R. Graig, G. A. Gray, D. J. Webb, and A. R. Butler, *Brit. J. Pharmacol.*, 1997, **122**, 1617.
53. I. L. Megson, S. Morton, I. R. Graig, F. A. Mazzei, R. A. Field, A. R. Butler, G. Caron, A. Gasco, R. Fruttero, and D. J. Webb, *Brit. J. Pharmacol.*, 1999, **126**, 639.
54. M. Sog, I. B. Wilkinson, H. MacCallum, S. Q. Khan, F. E. Strachan, D. E. Newby, I. L. Megson, and D. J. Webb, *Clin. Pharmacol. Ther.*, 2000, **68**, 75.
55. M. R. Miller, I. L. Megson, M. J. Roseberry, F. A. Mazzei, A. R. Butler, and D. J. Webb, *Eur. J. Pharmacol.*, 2000, **403**, 111.
56. I. L. Megson, M. Sog, F. A. Mazzei, A. R. Butler, J. C. Walton, and D. J. Webb, *Brit. J. Pharmacol.*, 2000, **131**, 1391.
57. A. R. Butler, H. H. Al-Sadoni, I. L. Megson, and F. W. Flitney, *Nitric Oxide: Biology and Chemistry*, 1998, **2**, 193.
58. M. R. Miller, M. J. Roseberry, F. A. Mazzei, A. R. Butler, D. J. Webb, and I. L. Megson, *Eur. J. Pharmacol.*, 2000, **408**, 335.
59. J. Gartwaite, E. Southam, C. L. Boulton, E. B. Nelsen, K. Schmidt, and B. Mayer, *Mol. Pharmacol.*, 1995, **48**, 184.

60. M. Feelish, P. Kotsonis, J. Siebe, B. Clement, and H. H. H. W. Schmidt, *Mol. Pharmacol.*, 1999, **56**, 243.
61. Z. Guo, J. Ramires, J. Li, and P. C. Wang, *J. Am. Chem. Soc.*, 1998, **120**, 3726.
62. Z. Guo, A. G. McGill, L. Yu, J. Li, J. Ramires, and P. C. Wang, *Bioorg. Med. Chem. Lett.*, 1996, **6**, 573.
63. G. Venturini, E. Fioravanti, M. Colasanti, T. Persichini, and P. Ascenzi, *Biochem. Mol. Biol. Int.*, 1998, **46**, 425.
64. M. Xian, X. Chen, Z. Liu, K. Wang, and P. G. Wang, *J. Biol. Chem.*, 2000, **275**, 20467.
65. U. K. Bandarage, L. Chen, X. Fang, D. S. Carvey, A. Glavin, D. R. Janero, L. G. Letts, G. J. Merser, J. K. Saha, J. D. Shroeder, M. J. Shumway, and S. W. Tam, *J. Med. Chem.*, 2000, **43**, 4005.
66. M. N. Muscaro and J. L. Wallace, *Am. J. Physiol.*, 2000, **276**, 1313.
67. I. S. Stamler, D. I. Simon, J. A. Osborne, M. E. Mulins, O. Jaraki, T. Michel, D. I. Singel, and J. Loscalzo, *Proc. Natl. Acad. Sci. USA*, 1992, **89**, 444.
68. C. Petit, V. Bernardes-Genisson, P. Hoffman, J.-P. Soushard, and S. Labidalle, *Braz. J. Med. Biol. Res.*, 1999, **32**, 1407.
69. *Annual Drug Data Rep.*, Ed. J. R. Prous, 1999, **21**, 800.
70. *Annual Drug Data Rep.*, Ed. J. R. Prous, 1999, **21**, 621.
71. *Annual Drug Data Rep.*, Ed. J. R. Prous, 1999, **21**, 39.
72. P. A. Serra, G. Esposito, M. R. Delogu, R. Migheli, G. Roghitta, E. Miele, M. S. Desole, and M. Miele, *Brit. J. Pharmacol.*, 2001, **132**, 941.
73. C. Daveu, C. Servy, M. Dendane, P. Marin, and C. Ducrocq, *Nitric Oxide: Biology and Chemistry*, 1997, **1**, 234.
74. D. R. Noble and D. L. H. Williams, *Nitric Oxide: Biology and Chemistry*, 2000, **4**, 392.
75. D. L. H. Williams, in *Methods in Ensymology*, 1999, **301**, Part C, Ed. L. Parker, Acad. Press, San Diego—London—Boston—New York—Sydney—Tokyo—Toronto, 299.
76. H. H. Al´Sadoni, I. Y. Khan, L. Poston, I. Fisher, and A. Ferro, *Nitric Oxide: Biology and Chemistry*, 2000, **4**, 550.
77. V. P. Reutov, E. G. Sorokina, V. E. Okhotin, and N. S. Kositsin, *Tsiklicheskie prevrashcheniya oksida azota v organizme mlekopitayushchikh* [*Cyclic Transformations of Nitric Oxide in the Mammal Body*], Nauka, Moscow, 1998 (in Russian).
78. V. I. Levina, A. V. Danilov, and N. B. Grigor´ev, *Khim.-Farm. Zhurn.*, 1995, No. 8, 55 [*Pharm. Chem. J.*, 1995, **29** (Engl. Transl.)].
79. H. Ishiropoulos, *Arch. Biophys. Biochem.*, 1998, **356**, 1.
80. K. Rehse and T. Shahrouri, *Arch. Pharm. Pharm. Med. Chem.*, 1998, **331**, 365.
81. M. D. Mashkovskii, *Lekarstvennye sredstva* [*Drugs*], issue 13, Torgsin, Khar´kov, 1997 (in Russian).
82. V. I. Levina, O. V. Azizov, A. P. Arzamastsev, N. B. Grigor´ev, and V. G. Granik, *Voprosy Biol., Med. i Farm. Khim.* (*Problems of Biol., Med., and Pharm. Chem.*), 2001, No. 1, 47 (in Russian).
83. R. Paccelli, T. Taira, J. A. Cook, D. A. Wink, and M. S. Krishna, *Lancet*, 1996, **347**, 900.
84. J. Jiang, S. I. Jordan, D. P. Barr, M. R. Gunther, H. Maeda, and R. P. Mason, *Molec. Pharmacol.*, 1997, **52**, 1081.
85. R. Camehn and K. Rehse, *Arch. Pharm. Pharm. Med. Chem.*, 2000, **333**, 27.
86. J. M. Fukuto, A. J. Hobbs, and L. J. Ignarro, *Biochem. Biophys. Research Communs*, 1993, **196**, 707.
87. F. N. Shirota, E. G. DeMaster, N. J. C. Lee, and H. T. Nagasawa, *Nitric Oxide: Biology and Chemistry*, 1999, **3**, 445.
88. S. B. King and H. T. Nagasawa, in *Methods in Ensymology*, 1999, **301**, Part C, Ed. L. Parker, Acad. Press, San Diego—London—Boston—New York—Sydney—Tokyo—Toronto, 211.
89. M. A. Sharpe and C. E. Copper, *Biochem. J.*, 1998, **332**, 9.
90. N. J. C. Lee, D. W. Shoeman, D. J. W. Goon, and H. T. Nagasawa, *Nitric Oxide: Biology and Chemistry*, 2001, **5**, 278.
91. M. N. Hughes and R. Cammack, in *Methods in Ensymology*, 1999, **301**, Part C, Ed. L. Parker, Acad. Press, San Diego—London—Boston—New York—Sydney—Tokyo—Toronto, 279.
92. L. N. Koikov, N. V. Alekseeva, E. A. Lisitza, E. S. Krichevsky, N. B. Grigoriev, A. V. Danilov, I. S. Severina, N. V. Pyatakova, and V. G. Granik, *Mendeleev Commun.*, 1998, 165.
93. L. N. Koikov, N. V. Alekseeva, N. B. Grigoriev, V. I. Levina, K. F. Turchin, T. Ya. Filipenko, I. S. Severina, I. K. Ryaposova, and V. G. Granik, *Mendeleev Commun.*, 1996, 94.
94. L. N. Koikov, N. V. Alekseeva, N. B. Grigor´ev, V. I. Levina, K. F. Turchin, T. Ya. Filipenko, M. D. Mashkovskii, M. E. Kaminka, V. B. Nikitin, G. N. Engalycheva, M. A. Kalinkina, E. S. Severina, I. K. Ryaposova, and V. G. Granik, *Khim.-Farm. Zhurn.*, 1995, **29**, No. 8, 56 [*Pharm. Chem. J.*, 1995, **29** (Engl. Transl.)].
95. J. Prous and J. Castacer, *Drug Future*, 1993, **18**, 9.
96. *Annual Drug Data Rep.*, Ed. J. R. Prous, 1995, **17**, 809.
97. M. Kato, S. Nishino, M. Ohno, S. Fukuyama, Y. Kita, Y. Harasawa, I. Makanishi, H. Tagasugt, and K. Sakane, *Bioorg. Med. Chem. Lett.*, 1996, **6**, 33.
98. K. Rehse, M. Herpel, and D. Piechocki, *Arch. Pharm. Pharm. Med. Chem.*, 1996, **329**, 83.
99. K. Rehse and T. Shahrouri, *Arch. Pharm. Pharm. Med. Chem.*, 2000, **333**, 157.
100. N. V. Pyatakova, N. B. Grigor´ev, and I. S. Severina, *Biokhimiya*, 1999, **64**, 111 [*Biochemistry (Moscow)*, 1999, **64** (Engl. Transl.)].
101. A. G. McGill, Y. Yang, J. Wang, L. Eshegoyen, and P. G. Wang, in *Methods in Ensymology*, 1999, **301**, Part C, Ed. L. Parker, Acad. Press, San Diego—London—Boston—New York—Sydney—Tokyo—Toronto, 235.
102. A. G. McGill, W. Zhang, J. Wittbrodt, J. Wang, H. B. Schlegel, and P. G. Wang, *Bioorg. Med. Chem.*, 2000, **8**, 405.
103. Y. Hou, W. Xie, A. J. Janczuk, and P. G. Wang, *J. Org. Chem.*, 2000, **65**, 4333.
104. Z. Guo, N. Miranda, and P. G. Wang, in *Methods in Ensymology*, 1999, **301**, Part C, Ed. L. Parker, Acad. Press, San Diego—London—Boston—New York—Sydney—Tokyo—Toronto, 249.
105. G. Ya. Shvarts, N. B. Grigor´ev, I. S. Severina, I. K. Ryaposova, A. S. Lapitskaya, L. B. Volodarskii, A. Ya. Tikhonov, I. F. Kerbnikova, D. G. Mazhukin, and V. G. Granik, *Khim.-Farm. Zhurn.*, 1994, **28**, No. 4, 38 [*Pharm. Chem. J.*, 1994, **28** (Engl. Transl.)].

106. I. S. Severina, I. K. Ryaposova, L. B. Volodarsky, D. C. Mazhukin, A. Ya. Tichonov, G. Ya. Schwartz, V. G. Granik, D. A. Grygoriev, and N. B. Grygoriev, *Biochem. Mol. Biol. Int.*, 1993, **30**, 357.
107. I. S. Severina, N. N. Belushkina, and N. B. Grygoriev, *Biochem. Mol. Biol. Int.*, 1994, **33**, 957.
108. I. S. Severina, N. N. Belushkina, and N. B. Grigor´ev, *Biokhimiya*, 1994, **59**, 1689 [*Biochemistry (Moscow)*, 1994, **59** (Engl. Transl.)].
109. D. I. Uterbergenov, V. V. Kramtsov, L. P. Vlasenko, A. L. Markel, D. C. Mazhukin, A. Ya. Tichonov, and L. B. Volodarsky, *Biochem. Biophys. Res. Commun.*, 1995, **214**, 1023.
110. I. A. Kirilyuk, D. I. Uterbergenov, D. C. Mazhukin, K. Fechner, K. Mertsc,V. V. Kramtsov, I. E. Blasig, and R. F. Haseloff, *J. Med. Chem.*, 1998, **41**, 1027.
111. K. Rehse and H. Muller, *Arch. Pharm.* (*Weinheim*), 1995, **328**, 765.
112. K. Schonafinger, *Farmaco*, 1999, **54**, 316.
113. R. Camehn and K. Rehse, *Arch. Pharm. Pharm. Med. Chem.*, 2000, **333**, 130.
114. I. J. Bhat, W. Clegg, H. Maskill, M. R. J. Elsegood, I. D. Menneer, and P. C. Meatt, *J. Chem. Soc., Perkin Trans. 2*, 2000, 1435.
115. D. C. Bohle and J. A. Jimonigie, *J. Org. Chem.*, 2000, **65**, 5685.
116. J. E. Saavedra, T. M. Dunams, J. L. Flippen-Anderson, and L. K. Keefer, *J. Org. Chem.*, 1992, **57**, 6134.
117. J. E. Saavedra, P. J. Shami, L. Y. Wang, K. M. Davies, M. N. Booth, M. L. Citro, and L. K. Keefer, *J. Med. Chem.*, 2000, **43**, 261.
118. L. K. Keefer, R. W. Nims, K. M. Davies, and D. A. Wink, *Methods in Ensymology*, 1996, **268**, Part C, Ed. L. Parker, Acad. Press, San Diego—London—Boston—New York—Sydney—Tokyo—Toronto, 281.
119. B. R. Jacobs, D. J. Smith, B. Zingarelli, D. J. Passerini, E. T. Ballard, and R. J. Brilli, *Nitric Oxide: Biology and Chemistry*, 2000, **4**, 412.
120. K. L. Homer and I. C. Wanstall, *Brit. J. Pharmacol.*, 2000, **131**, 673.
121. *Annual Drug Data Rep.*, Ed. J. R. Prous, 1997, **19**, 131.
122. *Annual Drug Data Rep.*, Ed. J. R. Prous, 1997, **19**, 177.
123. J. E. Saavedra, T. R. Billiak, D. L. Williams, Y.-M. Kim, S. C. Watkins, and L. K. Keefer, *J. Med. Chem.*, 1997, **40**, 1947.
124. P. Furnandez-Tomu, I. Lizasoqin, I. C. Lera, P. Lorenzo, and M. A. Moro, *Neuropharmacology*, 1999, **38**, 1307.
125. *Annual Drug Data Rep.*, Ed. J. R. Prous, 2000, **22**, 9999.
126. G. J. Southan and A. Srinivazan, *Nitric Oxide: Biology and Chemistry*, 1998, **2**, 270.
127. K. Rehse and M. Herpel, *Arch. Pharm. Pharm. Med. Chem.*, 1998, **331**, 104.
128. K. Rehse and M. Herpel, *Arch. Pharm. Pharm. Med. Chem.*, 1998, **331**, 111.
129. K. Rehse and M. Herpel, *Arch. Pharm. Pharm. Med. Chem.*, 1998, **331**, 79.
130. J. Prous and J. Castacer, *Drug Future*, 1993, **18**, 9.
131. V. I. Levina, O. V. Azizov, N. V. Pyatakova, O. G. Busygina, I. S. Severina, A. P. Arzamastsev, N. B. Grigor´ev, and V. G. Granik, *Voprosy Biol., Med. i Farm. Khim.*(*Problems of Biol., Med., and Pharm. Chem.*), 2001, No. 2, 54 (in Russian).
132. C. R. White, R. P. Patel, and V. D. Darley-Usmar, in *Methods in Ensymology*, 1999, **301**, Part C, Ed. L. Parker, Acad. Press, San Diego—London—Boston—New York—Sydney—Tokyo—Toronto, 288.
133. H. Kankaanranta, R. G. Knowles, P. Vuorinen, O. Kosonen, P. Holm, and E. Moilonen, *Molec. Pharmacol.*, 1997, **51**, 882.
134. A. Schrammel, S. Pfeiffer, K. Schmidt, D. Koesling, and B. Mayer, *Molec. Pharmacol.*, 1998, **54**, 207.
135. N. Paolossi, U. E. G. Ekelund, T. Isoda, M. Ozaki, K. Vandegaer, D. Georgakopoulos, R. W. Harrison, D. A. Kass, and J. M. Hare, *Am. Physiol. Heart. Circ. Physiol.*, 2000, **279**, 1982.
136. A. M. Sousa and W. A. Prado, *Brain Research*, 2001, **897**, 9.
137. M. Z. Asmawi, E. Moilanen, K. Annula, P. Rahkonen, and H. Kankaanranta, *Eur. J. Pharmacol.*, 1999, **378**, 123.
138. *Annual Drug Data Rep.*, Ed. J. R. Prous, 1991, **13**, 945.
139. *Annual Drug Data Rep.*, Ed. J. R. Prous, 1994, **16**, 250.
140. *Annual Drug Data Rep.*, Ed. J. R. Prous, 1995, **16**, 254.
141. *Annual Drug Data Rep.*, Ed. J. R. Prous, 1996, **17**, 815.
142. *Annual Drug Data Rep.*, Ed. J. R. Prous, 1996, **17**, 825.
143. M. Feelish, K. Schonafinger, and E. Noak, *Biochem. Pharmacol.*, 1992, **44**, 1149.
144. C. Medana, G. Ermondi, R. Fruttero, A. Di Stilo, A. Ferretti, and A. Gasco, *J. Med. Chem.*, 1994, **37**, 4412.
145. O. G. Busygina, N. V. Pyatakova, Yu. V. Khropov, I. V. Ovchinnikov, N. N. Makhova, and I. S. Severina, *Biokhimiya*, 2000, **65**, 540 [*Biochemistry (Moscow)*, 2000, **65** (Engl. Transl.)].
146. R. Ferreoli, G. C. Folco, A. Ferretti, A. M. Gasco, C. Medana, R. Fruttero, M. Cevelli, and A. Gasco, *Brit. J. Pharmacol.*, 1995, **114**, 816.
147. H. Bohn, J. Brendel, P. A. Martorana, and K. Schonafinger, *Brit. J. Pharmacol.*, 1995, **114**, 1605.
148. Li Mu, S.-s. Feng, and M. L. Go, *Chem. Pharm. Bull.*, 2000, **48**, 808.
149. *Annual Drug Data Rep.*, Ed. J. R. Prous, 1994, **16**, 551.
150. M. Barbinaria, G. Sorba, C. Medana, C. Cena, M. Adami, G. Morini, Z. Pazzoli, G. Coruzzi, and A. Gasco, *Helv. Chim. Acta*, 2000, **83**, 287.
151. *Annual Drug Data Rep.*, Ed. J. R. Prous, 1997,**19**, 999.
152. *Annual Drug Data Rep.*, Ed. J. R. Prous, 1996, **18**, 141.
153. G. Sorba, C. Medana, R. Fruttero, C. Cena, A. P. Stilo, U. Galli, and A. Gasco, *J. Med. Chem.* 1997, **40**, 463.
154. *Annual Drug Data Rep.*, Ed. J. R. Prous, 1999, **21**, 787.
155. N. B. Grigor´ev, G. V. Chechekin, A. P. Arzamaztsev, V. I. Levina, and V. G. Granik, *Khimiya geterotsikl. soedinenii*, 1999, № 7, 902 [*Chem. Heterocycl. Compd.*, 1999, No. 7 (Engl. Transl.)].
156. P. Cogolli, L. Testaferri, M. Tiecco, and M. Tingoli, *J. Chem. Soc., Chem. Commun.*, 1979, 800.
157. *Cancer Chemotherapeutic Agents*, Ed. W. O. Foye, A. C. F. Professional Reference Book, 1995, 698 p.
158. A. V. Danilov, O. B. Romanova, I. K. Sorokina, G. V. Chechekin, B. V. Tyaglov, N. B. Grigor´ev, and V. G. Granik, *Voprosy Biol., Med. i Farm. Khim.*(*Problems of*

Biol., Med., and Pharm. Chem.), 2001, No. 2, 50 (in Russian).
159. V. I. Levina, N. B. Grigoriev, V. G. Granik, *Intern. Pharm. Federation Congress, Abstrs. (Nice, France)*, 2002, p. 302.
160. V. I. Levina, A. V. Danilov, N. V. Pyatakova, I. S. Severina, N. B. Grigor´ev, and V. G. Granik,*IX Ross. Natsional´nyi kongress "Chelovek i lekarstvo"* [*IX Russ. National Congress "Man and Drugs"*], *Abstrs.*, Folium, Moscow, 2002, 650 (in Russian).
161. D. Labor and M. K. Mahunti, *Pol. J. Chem.*, 1986, **60**, 589.
162. N. F. Russo, M. Tomasz, M. Muller, and R. Lipman, *Mol. Pharmacol.*, 1977, **13**, 872.
163. J. Jin-Young and D. E. Berg, *Antimicrob. Agents Chemother.*, 2000, **44**, 3127.
164. R. J. Knox, R. C. Knight, and D. I. Edwards, *Biochem. Pharmacol.*, 1983, **32**, 2149.
165. R. P. Mason and P. D. Josephy, *J. Inorg. Biochem.*, 1985, **24**, 161.
166. K. Hofmann, in *The Chemistry of Heterocycl. Compounds — Imidazole and its Derivatives*, Interscience, New York, 1953, 135.
167. A. F. Cockerill, R. C. Harden, and D. N. B. Mallen, *J. Chem. Soc., Perkin Trans. 2*, 1972, 1428.
168. V. I. Levina, O. V. Azizov, N. V. Pyatakova, V. A. Parshin, I. S. Severina, A. P. Arzamastsev, N. B. Grigor´ev, and V. G. Granik, *IX Ross. Natsional´nyi kongress "Chelovek i lekarstvo"* [*IX Russ. National Congress "Man and Drugs"*], *Abstrs.*, Folium, Moscow, 2002, 649 (in Russian).
169. N. S. Kishani and Ho-L. Fung, in *Methods in Ensymology*, 1996, **268**, Part C, Ed. L. Parker, Acad. Press, San Diego—London—Boston—New York—Sydney—Tokyo—Toronto, 259.
170. L. A. Pevnitskii, *Vestnik Ross. Akad. Med. Nauk (Bull. Russ. Acad. Ned. Sci.*), 1996, No. 6, 43 (in Russian).
171. B. Bryune, K. Sandau, and A. von Kneten, *Biokhimiya*, 1998, **63**, 966 [*Biochemistry (Moscow)*, 1998, **63** (Engl. Transl.)].
172. A. Chicksman, *Biol. Rev.*, 1951, **26**, 59.
173. R. J. van´t Hof, P. K. Hocking, P. K. Wright, and S. H. Ralston, *Rheumatology*, 2000, **39**, 1004.
174. C. Napoli and L. J. Ignarro, *Nitric Oxide: Biology and Chemistry*, 2001, **5**, No. 2, 88.
175. T. Yamamoto and R. J. Bing, *Proc. Soc. Exp. Biol. Med.*, 2000, **223**, 200.
176. J. P. Eiserich, M. Hristova, C. E. Cross, A. D. Jones, B. A. Freeman, B. Halliweil, and A. Vliet, *Nature*, 1998, **391**, 393397.
177. D. A. Wink, I. Vodovoz, J. A. Cook, M. S. Krishna, S. Kim, D. Coffin, V. de Graff, A. M. Deluka, J. Liebmann, and J. B. Mitchell, *Biokhimiya*, 1998, **63**, 948 [*Biochemistry (Moscow)*, 1998, **63** (Engl. Transl.)].
178. H. Maeda, and T. Akaike, *Biokhimiya*, 1998, **63**, 1007 [*Biochemistry (Moscow)*, 1998, **63** (Engl. Transl.)].
179. J. Olesen and I. Jansen-Olesen, *Pathol. Biol.*, 2000, **48**, 648.
180. N. Toda, *Am. J. Physiol.*, 1990, **48**, 646.
181. M. Feelisch, *Naunyn-Schmiedeberg´s Arch. Pharmacol.*, 1998, **358**, 113.
182. U. K. Bandarage and D. R. Janero, *Mini-Reviews in Medicinal Chemistry*, 2001, 1.
183. S. Moncada, A. Higgs, and R. Furchgott, *Pharmacol. Rev.*, 1997, **49**, 137.
184. Zh.-K. Stökle, B. Myulle, R. Adriantsitokhaina, and A. Kleshchev, *Biokhimiya*, 1998, **63**, 976 [*Biochemistry (Moscow)*, 1998, **63** (Engl. Transl.)].
185. J. K. Ko and C. H. Cho, *Inflamm. Res.*, 1999, **48**, 471.
186. P. Kubes and J. L. Wallace, *Med. Inflamm.*, 1995, **4**, 397.
187. S. N. Elliott and J. L. Wallace, *J. Gastroenterol.*, 1998, **33**, 792.
188. S. W. Tam, J. K. Saha, D. S. Garvey, J. D. Schroeder, T. E. Shelekhin, D. R. Janero, L. Chen, A. Glavin, and L. G. Letts, *Inflammopharmacology*, 2000, **8**, 81.

Received November 9, 2001
in revised form March 4, 2002