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Exogenous nitric oxide donors and inhibitors of its formation (the chemical aspects)

V G Granik, S Yu Ryabova, N B Grigoriev

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Abstract. The published data on the biological role of nitric oxide formed *in vivo* by enzymic oxidation of L-arginine are generalised. **Special attention is given to exogenous nitric oxide donors, which can release NO** *in vitro* and *in vivo* in oxidation, reduction, or **hydrolytic cleavage. Also considered are the data on the chemical nature of inhibitors of NO-synthase responsible for nitric oxide formation from L-arginine. The bibliography includes 161 references.**

I. Introduction

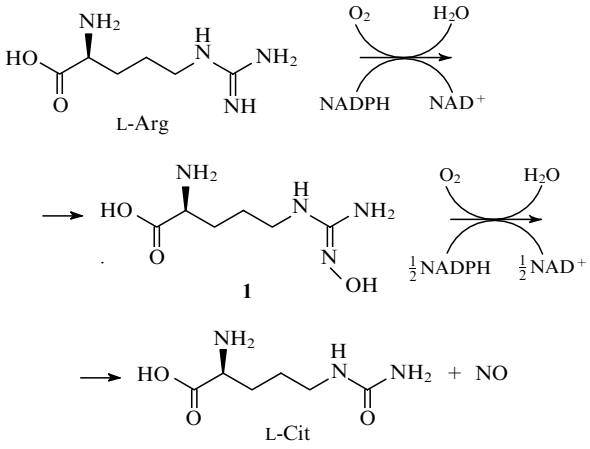
The discovery and verification of the mediator role of nitric oxide in living organisms have stimulated the search for drugs new in principle. Publications have appeared that were concerned primarily with the biological role of nitric oxide, problems of its insufficient or excessive release *in vivo,* and, correspondingly, the ways of increase or decrease in its content in organs and tissues.[1–10]

The present review generalises and considers the data on NO release from chemical compounds of different structure that can be combined under the common name of donors (or generators) of nitric oxide. In addition, the results of studies on inhibitors of isoforms of NO-synthase (NO-S) responsible for nitric oxide formation from L-arginine *in vivo* are discussed.

II. General aspects

Nitric oxide plays a crucial role in the functioning of the vascular system as an endogenous relaxant. The release of nitric oxide from endothelial cells of blood vessels results from an increase in the intracellular Ca^{2+} level, which initiates NO biosynthesis. Nitric oxide penetrates into neighbouring cells and activates soluble guanylate cyclase (SGC). The latter favours the transformation of guanosine triphosphate (GTP) into cyclic guanosine monophosphate (cGMP). The increase in the cGMP level in cells causes the relaxation of vessels, eventually resulting in the lowering of arterial pressure. Other important biological functions of nitric oxide are: inhibition of platelet aggregation and effects on the immune system as well as on the central and peripheral nervous systems. Hence, NO plays the key role in the control of vascular tonicity and neuromediator mechanisms and exerts a cytotoxic and cytostatic action. A wide variety of pathological states (*e.g.*, cardiovascular, infectious, and inflammatory diseases, brain injuries resulting from insults, *etc.*) may largely be due to the elevated or lowered content of nitric oxide.

Biosynthesis of NO in the organism occurs *via* the oxidation of L-arginine and is catalysed by NO-synthase (NO-S) (for NO-S isoforms see below) with the participation of reduced nicotinamide adenine dinucleotide (NADPH) as an electron donor. NO-S contains one mole each of (6*R*)-5,6,7,8-tetrahydrobiopterin (BH_4), flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), and heme per monomeric subunit as the cofactors. A general scheme of L-arginine oxidation is given below:[6]

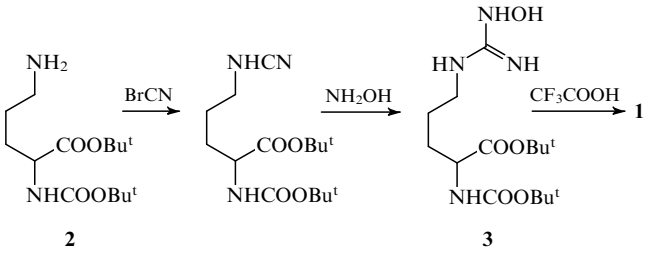


The oxidation occurs stereospecifically, L-arginine (but not its D-isomer) is the only source of nitric oxide.[11] L-*N*-Hydroxyarginine (**1**) is an intermediate product of L-arginine oxidation.[12–20] Compound **1** was synthesised [13] from the α,ω-diamino acid ester (**2**) *via* the *N*-cyano derivative, which was further converted into

V G Granik, S Yu Ryabova, N B Grigoriev
Centre for Drug Chemistry, Zubovskaya ul. 7, 119815 Moscow, Russian Federation. Fax (7-095) 246 66 33. Tel. (7-095) 246 07 49 (V G Granik), (7-095) 246 08 40 (S Yu Ryabova), (7-095) 120 88 29 (N B Grigoriev).

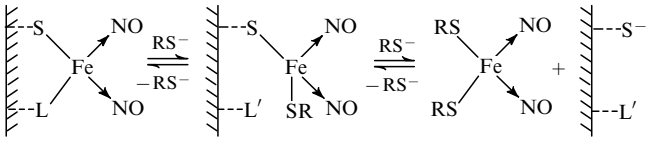
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the *N*-hydroxyguanidine intermediate (**3**). Deprotection of the carboxyl and α-amino groups resulted in *N*-hydroxyarginine **1**.



Like L-arginine, *N*-hydroxyarginine **1** causes the relaxation of vessels, which can be prevented by the NO-synthase inhibitors, N^{ω}-methyl- and N^{ω}-nitroarginines.[13] These data agree well with the finding that *N*-hydroxyarginine **1** is the intermediate in the syntheses of NO and L-citrulline (L-Cit) from L-arginine.[13] Special experiments [16,17] have shown that L-arginine oxidation by NO-synthase occurs in at least two stages. At the first stage, which requires the presence of NADPH and oxygen, *N*-hydroxyarginine **1** is formed; the second stage, which requires B-H_4, oxygen, and 0.5 equiv. of NADPH, gives L-citrulline and nitric oxide. The same authors have established that *N*-hydroxyarginine **1** is a good substrate for inducible NO-synthase (i-NO-S). It is necessary to note that the oxidation of N-hydroxyarginine **1** with a labelled ^{15}NOH-group results in $^{15}NO_2^-$ and $^{15}NO_3^-$ (the products of NO transformation), whereas the compound with a labelled ^{15}NH-group of the imidoxime fragment gives nonlabelled NO_2^- and NO_3^- anions. Consequently, i-NO-S utilises only the *N*-hydroxylated fragment for its oxidation. Using $H_2{}^{18}O$, it has been shown that L-citrulline formed upon the oxidation contains no label, *i.e.*, it is formed with the participation of O_2 rather than water. The idea that *N*-hydroxyarginine **1** is the intermediate in NO biosynthesis *in vivo* is in good accord with data on the pharmacological activity of this compound in comparison with the activity of arginine (including the experiments with NO-S inhibitors).[18] There is evidence [20] of the ability of compound **1** to undergo oxidation in cells to nitric oxide and nitrites also in the absence of NO-S, apparently *via* catalysis mediated by cytochrome P-450. Nevertheless, the main pathway of nitric oxide formation in the organism is the NO-S-catalysed oxidation of L-arginine. Two major isoforms of NO-S have been described in the literature, namely, the constitutive NO-synthase (c-NO-S) found in vascular endothelium and brain and inducible NO-synthase (i-NO-S) present in activated macrophages. The constituitive isoform requires Ca^{2+}/calmodulin for its activation and can be subclassified into endothelial and neuronal isoforms. The former generates NO that influences the lowering of arterial pressure and inhibits the aggregation of platelets; the latter (the neuronal enzyme) functions as a neurotransmitter. The main function of i-NO-S is the immune protection of the organism, *i.e.*, the synthesis of NO, a cytotoxic and antiinflammatory agent. Unlike c-NO-S, i-NO-S does not require regulation by Ca^{2+} (see Refs 10, 21).

Of great importance is the further fate of nitric oxide in living organisms, namely, its involvement in various biochemical processes and the mode of its deposition in cells. Although in many studies the role of a vasodilating agent has been ascribed to nitric oxide, the identity of NO with the so-called endothelium-derived relaxation factor (EDRF) is very doubtful due to the high reactivity of nitric oxide and, correspondingly, the high probability of its rapid biotransformation in living organisms. The fact that, unlike EDRF and *S*-nitrosothiols, NO enters into reaction with hemoglobin (Hb) to give a HbNO complex (data from the tracer technique, EPR spectroscopy), allowed one to conclude that EDRF is not free nitric oxide but rather is an *S*-nitrosothio compound of the *S*-nitrosocysteine type.[22] *S*-Nitrosothiols have been proposed as candidates for EDRF in a number of other investigations.[2,23–25] However, there exists an alternative viewpoint,[26–31] according to which EDRF is represented by dinitrosyl complexes of non-heme iron (DNCI) with low-molecular-weight thiol ligands. Both types of compounds (nitrosothiols and DNCI) have been detected in cells and tissues that generate nitric oxide from L-arginine under catalysis by NO-S.[29,30] The same authors have demonstrated that these compounds can undergo interconversions, *i.e.*, the deposition of nitric oxide according to both these mechanisms is not mutually exclusive. Protein ligands seem to have greater affinity for Fe(NO)-groups; however, in the interaction with thiols, such as cysteine and acetylcysteine, the following equilibrium is established:[29]



////////// – protein.

According to Vanin *et al.*,[29] protein DNCI provide stable storage of nitric oxide, whereas low-molecular-weight DNCI act as carriers. In endothelial cells, EDRF is largely represented by DNCI, whereas in the intercellular medium, by *S*-nitrosocysteine.[30] Since DNCI are much more stable than *S*-nitrosothiols, in animal organisms the equilibrium is markedly shifted to the left and the viewpoint, according to which EDRF is predominantly represented by iron complexes, appears to be rather convincing.[31]

$$\text{DNCI} \rightleftharpoons \text{RSNO}\,.$$

The binding of nitric oxide by iron seems to determine also the activation of soluble guanylate cyclase (SGC) responsible for GTP transformation into cGMP and the biochemical and biological effects produced thereby. It has been found [32] that the UV spectrum of SGC changes under the influence of NO. Simulation of this system has revealed that after addition of nitric oxide protoheme-CO complexes transform into the pentacoordinated Fe(II)-NO complexes that apparently mimic the behaviour of SGC. It is assumed [33] that NO nitrosylates heme-SGC to give a nitrosyl–heme complex, in which iron is out of the plane of the porphyrin ring. The structure of the complex becomes similar to that of the SGC activator, protoporphyrin IX, and SGC activation takes place. The NO influence on SGC having been established, the activation of this enzyme by several nitric oxide complexes with transition metals has been studied.[34] Three types of nitroso complexes have been examined: sodium nitroprusside, $Na_2[FeNO^+(CN)_5]$, in which the NO group bears a positive charge (nitrosonium cation), $K_3[CrNO(CN)_5]$ and $[CoNO(NH_3)_5]SO_4$ (where NO is the neutral group), and $K_3[CoNO^-(CN)_5]$, where NO is coordinated as the anion. The most effective activator of SGC is sodium nitroprusside whose activating effect is determined by the interaction of NO^+ with the heme of the enzyme. Nitroso complexes with cobalt and chromium, in which NO is a neutral group, activate SGC insignificantly, whereas the complexes with the NO^- anion inhibit the enzyme. In contrast to nitroprusside, other nitroso complexes have no hypotensive effect mediated by a heme-dependent mechanism.

Before we come to the consideration of the material concerning the analysis of various types of structures that are responsible for the release of exogenous nitric oxide or inhibit its formation in living organisms, it seems expedient to consider in this section the analytical methods allowing the identification of NO.

The main techniques (chemiluminescent, electrochemical, EPR, spectrophotometrical, chromatographic, electrophoretic, and fluorimetric) used to determine nitric oxide have been described in detail in a recent review.[8] Here it seems appropriate to cite some papers not mentioned in this review. Thus NO has been determined colorimetrically as nitrite anion and by EPR spectroscopy as the nitrosyl–ferrohemoglobin paramagnetic

complex obtained by decomposition of thionitrites in the presence of oxyhemoglobin.[35] Analysis of NO has been performed by EPR spectroscopy of an iron–mononitrosyl–dithiocarbamate complex.[15,28,36] A chemiluminescent method has also been used.[37,40] Special reference should be made to an indirect electrochemical method recently developed for the determination of nitric oxide.[41–45] It turned out that NO can be identified polarographically, since it is reduced on a mercury dropping electrode (MDE) in acidic media (pH < 3) to give an irreversible five-electron wave.[41]

$$NO + 5e^- + 6H^+ \longrightarrow NH_4^+ + H_2O.$$

The wave appears at −0.77 V (relative to SCE, saturated calomel electrode) and rapidly disappears when nitrogen is passed through. This does not allow one to consider this method as an analytically applicable one. It is more appropriate to detect NO from the reduction current of NO_2 that is formed upon the interaction of NO with dissolved atmospheric oxygen. In this case, the determination is carried out at a pH close to the physiological one.

$$2NO + O_2 \longrightarrow 2NO_2,$$

$$NO_2 + NO + 2OH^- \longrightarrow 2NO_2^- + H_2O.$$

In weakly acidic solutions (pH 3–4) a non-dissociated HNO_2 is formed, which is discharged on the MDE *via* a one-electron mechanism ($E_{1/2} = -1.0$ V *vs* SCE).

$$NO_2^- + H^+ \longrightarrow HNO_2,$$

$$HNO_2 + e^- \longrightarrow NO + {}^-OH.$$

The appearance of this wave is an indirect marker of the presence and concentration of NO in the solution. Yet, another possible method for the determination of nitric oxide is the introduction into the solution of polyvalent cations (La^{3+}, Ce^{3+}, and Nd^{3+}).[41] In this case, a wave with $E_{1/2} = -1.2$ V is observed.

$$NO_2^- + 4e^- + 4H_2O \longrightarrow NH_2OH + 5OH^-.$$

To further improve this method, a polarographic procedure [42–45] has been developed for the determination of NO in the form of the nitroprusside anion, which is formed upon the reduction of the compound under study with potassium ferrocyanide or upon its oxidation with potassium ferricyanide. For example, in the reaction of well-known NO generators, such as nitrates, with the ferrocyanide anion the following processes take place:

$$RONO_2 + [Fe(CN)_6]^{4-} + H^+ \longrightarrow ROH + HNO_2 + [Fe(CN)_6]^{3-},$$

$$HNO_2 + [Fe(CN)_6]^{4-} \longrightarrow [Fe(CN)_5NO]^{2-} + CN^- + OH^-.$$

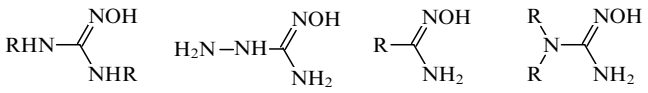
This interaction gives a polarographically active nitroprusside anion, and, in the appropriate selection of optimum conditions, this process can be used for reliable quantitation of nitric oxide.[42–44] Another analytical procedure [42,45] is based on a time-dependent decline of the anodic wave of cysteine in the presence of a nitric oxide source.

The data presented above point to the expediency of a search for compounds that can increase or decrease the nitric oxide content in organs and tissues, while the existence of reliable analytical methods for the determination of NO makes it possible to select the most promising NO donors *in vitro*. Below we consider methods of synthesis of NO donors and provide ample data on nitric oxide generators and inhibitors of its NO-S-catalysed *in vivo* synthesis and discuss some biological and toxicological problems arising from the effect of exogenous compounds on various biological systems.

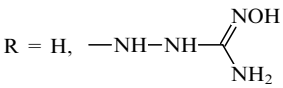
III. Exogenous donors of nitric oxide

1. Guanidines and related compounds

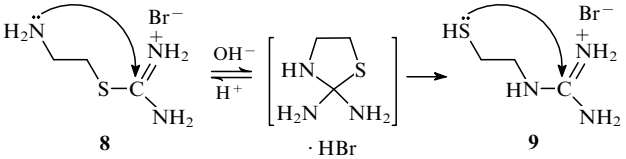
As indicated above, it is the guanidine fragment of L-arginine, the target for NO-S, that undergoes oxidation to the *N*-hydroxy derivative **1** and further to citrulline and nitric oxide. It is supposed that the binding of substrates with NO-S occurs also *via* the amino-acid part, but largely it is due to the guanidine fragment (with the heme of the enzyme and a special site of 'guanidine binding').[6] It could therefore be expected that some compounds having guanidine or guanidine-like groups can act as substrates for NO-S and can be transformed *via* the oxidative NO-synthase mechanism (including NO-S catalysis) with the evolution of NO. First of all, this applies to the main endogenous substrate, L-arginine, since enzymic processes are mostly highly specific. Of the investigations concerning arginine itself, special mention should be made of a study that has demonstrated that this amino acid and the ethyl ester of N^{α}-benzoyl-L-arginine can lower intraocular pressure *in vivo*.[46] In addition, L-arginine and its derivatives with lipophilic substituents can be used in the treatment of ischemia retinae.[47] The authors of these two papers believe that these phenomena are due to the ability of L-arginine and its derivatives to undergo oxidation with the liberation of nitric oxide and, correspondingly, cause the relaxation of blood vessels resulting in the lowering of blood pressure. The vasodilating effect of *N*-hydroxyarginine **1** is enhanced during its interaction with nitric oxide. It was shown that the reaction of compound **1** with NO, HNO_2, and other nitrosating agents gives an adduct able to cause the relaxation of vessels.[19] The reaction of certain cyanamides, carbodiimides, and other compounds with hydroxylamine resulted in a series of the corresponding *N*-hydroxyguanidines **4**–**7**.[19] These compounds also give adducts with nitrosating agents that liberate nitric oxide in aqueous solutions (data from chemiluminescent analysis).[19]



4 **5** **6** **7**

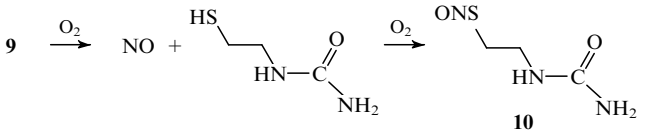


The structure of these adducts has not been established; however, it has been shown that they are stable prior to dissolution. In aqueous solutions, they decompose at different rates, the decomposition being irreversible at low pH (1.0–1.5). In organic solvents, the degradation occurs rapidly but the yield of NO is small. Using labelled nitrosating agents, it has been demonstrated that ^{15}NO incorporates into the adduct and it is this nitrogen (^{15}N) that is lost upon decomposition. Pharmacological tests have revealed the relaxation of vessels under the influence of these compounds. In this case, more labile adducts give a short-term and sharp relaxation, whereas slowly decomposing adducts elicit a more prolonged (on a time scale) response of lower intensity. It is self-evident that apart from the NO formed by degradation of its adducts with hydroxyarginine **1** and some other hydroxyguanidines, the NO formed by the oxidation of the guanidine fragment of arginine is also released. The β-aminoethylisothiouronium salt **8**, which readily rearranges into β-mercaptoethylguanidine **9** at pH 7.0, has been studied.[48,49]



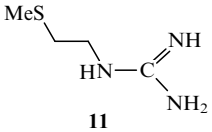
8 · HBr **9**

The equilibrium ratio **8** : **9** has been quantitatively estimated by the polarographic method.[49] It has been found that the amine **8** is completely converted into the thiol **9** at pH 7.0. This finding has led the authors to assume the probability of the same transition *in vivo*. It was conjectured that the oxidation of **9** with the formation of NO is accompanied by the capture of NO by the thiol group to give the *S*-nitrosothiol **10,** and thus the deposition of the released nitric oxide takes place.



10

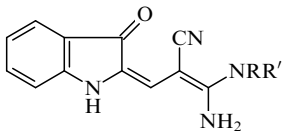
To test this hypothesis, β-methylthioethylguanidine **11** has been synthesised.



11

It turned out that the guanidine **11** activates SGC far less efficiently than thiol **9** and produces a less pronounced hypotensive effect, which probably confirms the expediency for a search for systems containing 'traps' capable of retaining and preserving nitric oxide for some time along with fragments responsible for NO release. This question will further be discussed in the consideration of other NO generators. It is worth special note that the oxidation of the thiol **9** seems to occur *via* the same mechanism as that of L-arginine, *i.e.*, with the participation of NO-S. Evidence in favour of this conclusion can be derived from the fact that SGC is not activated when the thiol **9** is used in combination with the NO-S inhibitor, N^{ω}-methylarginine.

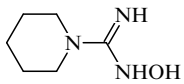
Yet, another new type of NO-donor is represented by dienediamines of the indolinone series **12**.[50, 51]



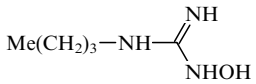
12

Compounds **12** form nitric oxide upon oxidation and, depending on the substituent at the nitrogen atom, manifest a more or less pronounced ability to activate SGC and exert hypotensive activity *in vivo*.

The chemical oxidation of *N*-hydroxyguanidines, such as **1**, **13**, and **14,** which is accompanied by NO release, has been studied.[15] Of the oxidants tested only two, *viz*., lead tetraacetate and potassium ferricyanide/hydrogen peroxide, have been found to direct the process along the desired pathway.

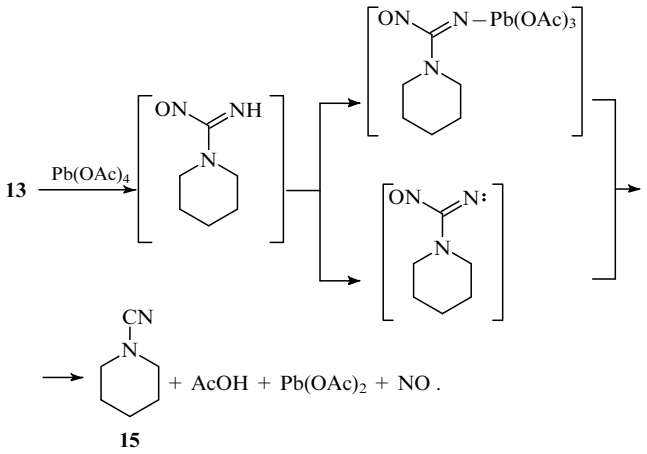


13



14

N-Cyanopiperidine **15** has been isolated and identified as one of the oxidation products of compound **13**. A scheme of the process that differs basically from the enzymic oxidation of L-arginine and *N*-hydroxyarginine has been proposed:[15]

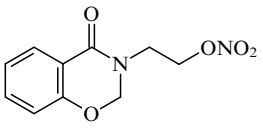


15

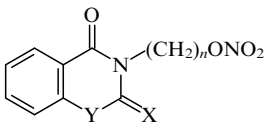
Interestingly, chemical oxidation does not result in ureas, *i.e.*, products similar to citrulline formed upon arginine oxidation. This fact provides additional evidence in favour of the enzymic pathway of the *in vivo* oxidation of guanidine derivatives. It is noteworthy that oxidation by other reagents results in HNO which dimerises to give HON = NOH. The decomposition of the latter gives N_2O and H_2O.

2. Nitrates

For the derivatives containing the guanidine or modified guanidine fragment, oxidation is the main pathway of NO generation. On the contrary, the formation of nitric oxide from nitrate vasodilators (drugs of the nitroglycerin type that have been used for many years) and related compounds requires reduction. It is now obvious that the coronarodilator effect of nitrates is associated with enhanced release of nitric oxide by these donors.[37, 38, 52–58] Nitrates are traditionally used in the treatment of stenocardia but their clinical applications can further be extended to diabetes mellitus, atherosclerosis, and vasospasm. In addition to the well-known nitrates,[37, 38, 52] some authors offer novel compounds, such as nitroxyalkyl coumarins.[53] The activity of some representatives of this series exceeds that of nitroglycerin 2- to 10-fold. A recent report [54] on a novel drug, the benzoxazine derivative **16,** points to an improved action on coronary vessels. This drug is undergoing the second stage of clinical trials. Some novel oxazine, thiazine, and pyrimidine derivatives **17** manifest antiischemic activity.[54]



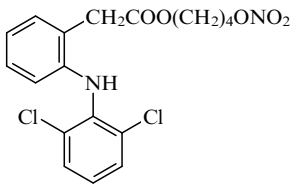
16



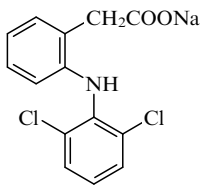
17

Y = O, NH, S; X = O, S; *n* = 2, 3

Data have been given [55] on the vasodilating and antiaggregating activity of NO donors, such as *S*- and *N*-nitroso compounds, relative to nitroglycerin. The vasodilating action of nitroglycerin is at a sufficiently high level; however, with regard to the inhibition of platelet aggregation this drug is strongly inferior to *S*- and *N*-nitroso compounds. Nitrofenac (diclofenac δ-nitroxybutyl ester, **18**)[57] belongs to novel NO donor compounds.



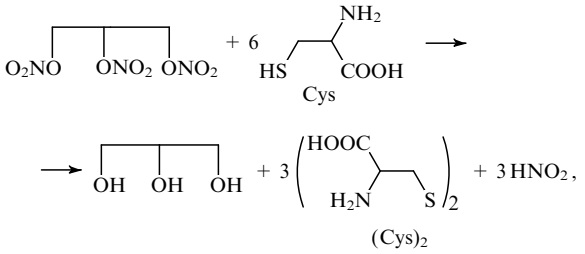
18



Diclofenac sodium
(voltaren, orthofen)

With regard to antiinflammatory and antipyretic action, nitrofenac is similar to diclofenac sodium (voltaren, orthofen) but its antithrombotic activity (which is characteristic of NO donors) is much higher. However, the main advantage of **18** in comparison with diclofenac sodium is its ability to markedly reduce gastrotoxicity without any detriment to its ability to inhibit prostaglandin synthesis. This important property is ascribed to the presence of a nitrate fragment in compound **18**.

Direct evidence in favour of NO formation from nitrates in the presence of thiols has been obtained using the polarographic method.[45] The course of the reaction of nitroglycerin with thiols was followed by the decline of the anodic waves of thiol and nitrate. Thus, the polarographic wave reflecting cysteine oxidation gradually declines with time according to the equation:



Cys

$(Cys)_2$

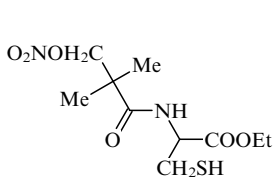
$$HNO_2 \longrightarrow NO + H_2O.$$

It has been established that nitroglycerin reduction with cysteine or unithiol (sodium 2,3-dimercaptopropane sulfonate) occurs in two steps, *i.e.*, reductive hydrolysis of the nitrate with thiol and reduction of the NO_2^- formed to NO. Disproportionation of HNO_2 is also possible:

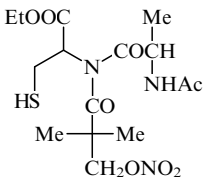
$$3HNO_2 \longrightarrow HNO_3 + 2NO + H_2O.$$

The rate of the nitroglycerin reaction with thiols is rather low, being essentially less than the manifestation of the therapeutic effect of this drug, which suggests the enzymic formation of nitric oxide from nitroglycerin *in vivo*.[45] One of the most important problems in the clinical application of nitrates is the development of a tolerance effect, which is believed to be associated with different mechanisms of blockade or metabolism of nitroglycerin and/or with the effect of its active metabolite, nitric oxide.[58] In this case tolerance is not eliminated with an increase in the nitroglycerin dose. A proposal has been put forward [59] that tolerance is caused by rapid depletion of intracellular depots of cysteine or, in more general terms, by the deficiency of SH-containing compounds. This hypothesis has given rise to a noticeable trend in the synthesis of SH-containing NO donors. In this case 'intramolecular assistance' is possible (as in the case of β-mercaptoethylguanidine) consisting in intramolecular trapping of the released NO to give rather unstable *S*-nitrosothiols, which serve as a source of NO in the organism. Synthetic compounds based on this principle are highly effective vasodilators, potent nitric oxide donors, and SGC activators. Among them, compound **19** exerts the highest activity.[59]

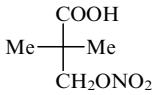
Apparently, compound **19** and its analogues do not produce the effect of development of tolerance. Synthesis of the ethyl ester of [*N*-(3-nitroxypivaloyl)-*N*-(*N'*-acetyl-DL-alanyl)-L-cysteine (SPM-5185, **20**), a coronarodilator drug comparable in activity with nitroglycerol, has been described.[60–63]



19



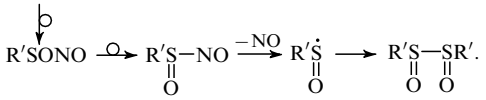
20



21

To check the idea that the presence of a thiol group in the molecule of nitrate is necessary to exclude the development of tolerance, a comparative study of the biological effects of compounds **20** and **21** has been carried out. It has turned out that the activity of the former markedly exceeded that of the latter, which was devoid of the thiol group; however, neither of these compounds induces any noticeable tolerance. This finding made it possible to assume [61] that the cysteine fragment of compound **20** is not necessary and the reduction of the number of thiol groups can hardly be the reason for tolerance. The fact that tolerance did not develop with ester **20** has been specially examined and confirmed.[64] However, in another recent study [65] it has been demonstrated again that it is particulary the depletion of thiol groups that is the true reason for the development of tolerance to nitrates. Bron *et al.*[65] have synthesised compounds containing ONO_2^- groups together with a thiazolidine fragment whose hydrolysis gives free thiol compounds. Pharmacological studies of these compounds have made it possible to conclude that the presence of the released nitric oxide together with the liberated thiol groups in the system prevents nitrate tolerance. In conclusion, let us return to the reaction of nitrates with thiols which, as can be evidenced from the above data, is the most important mechanism of their biological action. The reaction of thiols with nitrates has been analysed [9] and it has been established that the thiol structure is the crucial factor. All thiols decompose nitrates to the nitrite anion, but only some of them (cysteine, *N*-acetylcysteine) convert them into nitric oxide. If NO is formed (even together with NO_2^-), the activation of SGC occurs; if only NO_2^- is formed, no activation of SGC takes place. These differences in the behaviour of thiols were rationalised in terms of a possible intramolecular rearrangement (thionitrate $\longrightarrow$ sulphenyl nitrite).

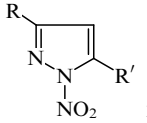
$$RONO_2 + R'SH \longrightarrow ROH + R'SNO_2,$$
$$R'SNO_2 + R'SH \longrightarrow R'SSR' + HNO_2,$$



Obviously, the validity of the concepts concerning nitrate metabolism needs further verification and analysis.

3. Other nitro-, nitroso-, and related compounds

Like O-nitro, N-nitro derivatives can also be generators of nitric oxide, which can be exemplified by the study of nitropyrazoles ($PzNO_2$, **22**).[66]



22

Both electrochemical and chemical reduction of these compounds with potassium ferrocyanide or cysteine gives nitric oxide. Compounds **22** activate SGC.The putative mechanism of this transformation is given below, using the reaction with potassium ferrocyanide as an example:

$$PzNO_2 + 3K_4[Fe(CN)_6] + 2H_2O \longrightarrow$$
$$\longrightarrow K_2[Fe(CN)_5NO] + 2K_3[Fe(CN)_6] + KCN + 3KOH + PzH.$$

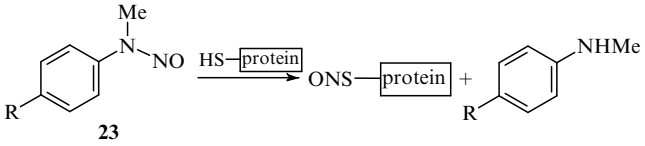
With thiol compounds (cysteine), pyrazoles **22** react according to the scheme:

$$PzNO_2 + 2RSH \longrightarrow PzH + RSSR + HNO_2,$$

$$RSH + HNO_2 \longrightarrow RSNO + H_2O,$$

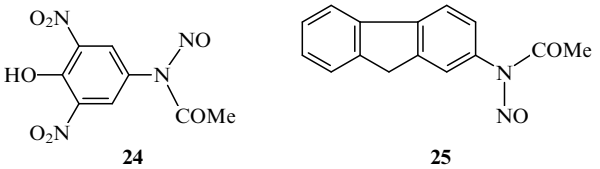
$$2RSNO \longrightarrow RSSR + 2NO.$$

A number of investigations have been dedicated to the analysis of compounds containing an *N*-nitroso group. A series of *N*-methyl-*N*-nitrosoanilines **23**, which, as it turned out, were able to *S*-nitrosate cysteine-containing compounds (*e.g.*, papain) and thus form stable NO-depots, have been investigated.[67]



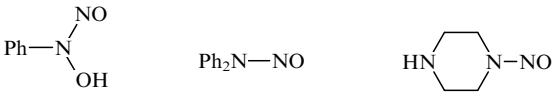
23

N-Nitroso compounds **24** and **25**, which exhibit a pronounced mutagenic effect, manifest vasodilating properties; the vascular response can be prevented by hemoglobin that binds NO or by Methylene Blue that inhibits guanylate cyclase.[68]



24 **25**

Interestingly, compounds **24** and **25** can deaminate DNA bases. It is assumed [68] that the above effects are due to the release of nitric oxide. A group of *N*-nitroso compounds **26**–**28** acting as nitric oxide donors have been studied.[36]



26 **27** **28**

For *N*-nitroso-*N*-hydroxyaniline **26,** an oxidation reaction with involvement of oxyhemoglobin **29** has been suggested.

$$HbO_2 + PhN(O^-)NO \longrightarrow HbO_2^{\bullet -} + PhN(NO)O^{\bullet},$$

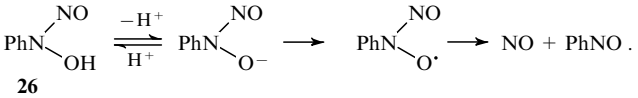
29

$$PhN(NO)O^{\bullet} \longrightarrow PhNO + NO,$$

$$HbO_2^{\bullet -} + 2H_2O \longrightarrow MtHb + H_2O_2 + OH^-.$$

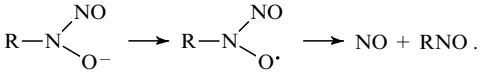
Methemoglobin

The scheme of oxidation of compound **26** without the involvement of hemoglobin is given below:[69]



26

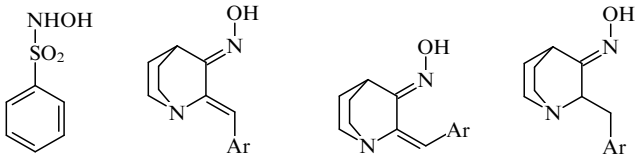
For compounds of the type **26,** a peroxidase-catalysed enzymic oxidation is observed. The oxidation can also be modelled electrochemically.[70]



In both cases nitric oxide is formed.

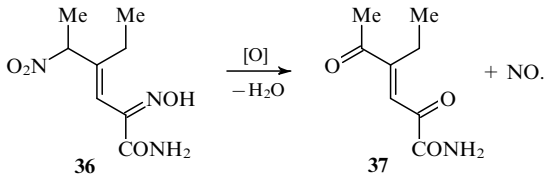
Some studies have dealt with the analysis of derivatives of *N*-nitroso-*N*-alkoxy compounds.[69–71] Thus compounds RN(NO)OR′ (**30**: R = R′ = Me; **31**: R = R′ = Et) can effectively generate nitric oxide, which may be the reason for their far greater cancerogenic activity [71] in comparison with, *e.g.*, *N*-nitrosodimethylamine.

These studies are close to those on the oxidation of compounds with the N–O bond.[72–76] This group includes nitrones giving nitric oxide upon photolysis [73] and activating SGC, nitroxyl (HNO) producing biological effects similar to those of NO,[74] peroxynitrite ($ONOO^-$), which gives NO upon interaction with glucose and causes the relaxation of vessels inhibited by oxyhemoglobin,[75] and *N*-hydroxybenzenesulfamide **32**,[76] Piloti's acid, which upon oxidation releases NO (presumably this process occurs *via* an enzymic route).[76] Oximes (*e.g.*, **33**–**35** derived from quinuclidone-3) have turned out to be interesting objects for the study of nitric oxide generation.



32 **33** **34** **35**

Using an indirect electrochemical method [$K_3Fe(CN)_6$ and $K_4Fe(CN)_6$, see above], it has been established that the majority of these compounds generate NO upon oxidation.[44,77] The highest yield of nitric oxide has been observed for compounds **34** and **35** (in both cases, Ar is *o*-hydroxyphenyl); *i.e.*, the proximity of the oxime fragment and the phenolic hydroxyl favours the formation of NO. It is essential that these compounds are oxidised to NO even by atmospheric oxygen. These oximes activate SGC and have hypotensive action. A conclusion has been made [44] about the possible role of the phenolic *ortho*-hydroxyl group as nitric oxide carrier, which stabilises NO in the form of *O*-nitrites or *O*-nitrates. The interaction of NO with phenolic antioxidants and phenoxyl radicals that can be regarded as nitric oxide carriers in biological systems is documented.[78] The oxidation of compound **36** containing both the oxime fragment and the nitro group has been studied.[79]



36 **37**

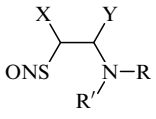
At pH 7.5, compound **36** rapidly transforms into the diketone (**37**) with the release of NO. The latter has been identified by EPR spectroscopy, colorimetric titration, and using a procedure based on the interaction with the tyrosyl residue of ribonucleotide reductase. Compound **36** is a potent vasodilating agent, it activates SGC and increases the level of intracellular cGMP. This study provides no direct data as to which of the groups (nitro group, oxime group, or both) make the main contribution to NO production.

There is experimental evidence that 5-nitrofurans and 5-nitroimidazoles form nitrosyl complexes of hemoglobin in the presence of ascorbic acid as reducing agent, *i.e.*, they released nitric oxide.[80] In conclusion, it is appropriate to mention that NO is formed in the photolysis of 2-methyl-2-nitrosopropane.[81] Upon irradiation, this compound induces the relaxation of vessels, activates SGC, and, correspondingly, increases the cGMP level in biological solutions.

4. *S*-Nitrosothiols

Since *S*-nitrosothiols represent, perhaps, the most abundant and especially well-studied group of exogenous generators of nitric

oxide, it seems expedient to isolate them as a separate class of NO donors. Roy *et al.*[35] have proposed the main requirements for nitric oxide generators that confirm the expediency of a search for new drugs particularly in the *S*-nitrosothiol series. The reasons for this are: (i) simplicity of synthesis and the possibility of isolation of pure compounds in a stable (predominantly solid) form and (ii) capability for relatively fast degradation in aqueous solutions at physiological pH resulting in the release of NO without any redox activation. This decomposition occurs with quantitative liberation of NO and is not accompanied by the formation of toxic side products. A large group of *S*-nitroso compounds of the general formula **38** have been synthesised.[35]



38

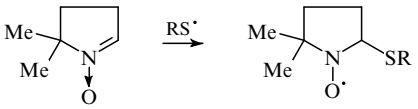
The stability of these compounds in solution has been studied, and their ability to generate nitric oxide was confirmed by several known methods. Quantitative determination of the half-life of *S*-nitrosothiols **38** in a phosphate buffer at pH 7.0 has been carried out: they vary from several minutes to tens of hours depending on the structure. It is believed that the formation of NO is based on homolytic cleavage of the S—N bond.

$$2RSNO \longrightarrow 2RS^{\cdot} + 2NO,$$

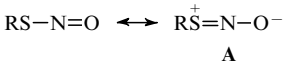
$$2RS^{\cdot} \longrightarrow RSSR,$$

$$4NO + O_2 + 2H_2O \longrightarrow 4HNO_2.$$

The production of NO is accompanied by the formation of the RS· radical, which is so unstable that it cannot be detected by EPR spectroscopy. If 5,5-dimethylpyrroline *N*-oxide is used as a trap, its reaction with the thyil radicals yields stable paramagnetic species that can be detected by EPR spectroscopy.[35]

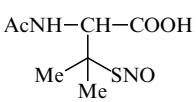


The effect of structure on the stability of nitrosothiols has been ascribed to the contribution of the resonance structure **A** stabilising the molecule with respect to the homolytic cleavage of the S—N bond. The contribution of this structure increases with an enhancement of the electron-donor properties of the radical R (in these cases the stability of *S*-nitrosothiols increases also).

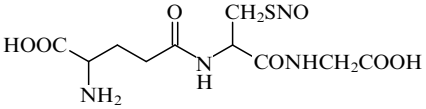


A

Thiyl radicals have been discussed by Feelish,[9] who has demonstrated potential hazardous properties of these highly reactive compounds. They can not only undergo dimerisation, but also enter into other reactions, thereby giving compounds with unknown biological activity. Another potential danger from the use of *S*-nitrosothiols is the reaction of transnitrosation of SH groups of proteins. This reaction may cause irreversible modification of SH groups of enzymes and result in the formation of mixed disulfides with a partial or complete loss of enzymic activity. The problem of transnitrosation has been discussed in detail by Butler *et al.*,[82] who have analysed the properties of *S*-nitroso-*N*-acetylpenicillamine **39** and *S*-nitrosoglutathione **40**.

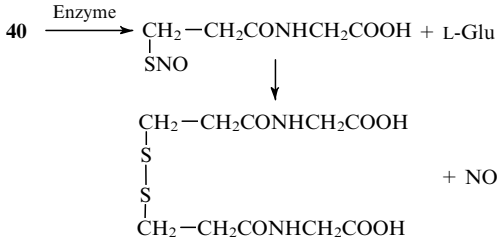


39

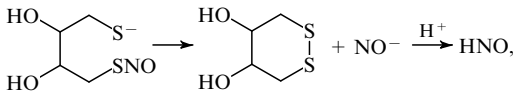


40

Compounds **39** and **40** differ essentially in their chemical properties. The former is unstable; its decomposition to the corresponding disulfide and NO is catalysed by Cu^{2+}. The latter is stable in solutions over many hours and are not affected by copper cations. Dicks[83] has pointed to the long induction period preceding the decomposition of compound **40** rather than its complete stability under these conditions. Nevertheless, *S*-nitrosoglutathione **40** is comparable in activity towards vascular relaxation with compound **39** and is more active as a platelet aggregation inhibitor. Compound **40** rapidly reacts with cysteine to give *S*-nitrosocysteine, an unstable compound, which rapidly generates nitric oxide.[82] Thus, *S*-nitrosoglutathione **40**, which is relatively stable in solution, can produce NO at a high rate in the presence of cysteine. An alternative (and, seemingly, also fast) pathway of NO release from compound **40** is the enzymic pathway mediated by γ-glutamyl transpeptidase.



According to Park,[84] transnitrosation is the primary mechanism of the hypotensive effect of *S*-nitrosothiols. This viewpoint can be validated by the much lower rate of their hydrolysis relative to the development of the pharmacological effect. As for transnitrosation, it is a rather fast process. The same conclusion has been made by Mathews and Kerr.[85] Neuroprotective effects of *S*-nitrosothiols are caused by transnitrosation and, as stated by Arnelle and Stamler,[86] these processes occur with the participation of NMDA receptors (NMDA — *N*-methyl-D-asparagine). At the same time, neurodestructive effects of NO are probably due to its interaction with the superoxide anion (O_2^-) resulting in peroxynitrite. It is conjectured[86] that the vasodilating, antiaggregating, and antimicrobial effects of *S*-nitrosothiols do not depend on the rate of NO release. Arnelle and Stamler[86] have considered the possibility of formation of such species as NO, NO^+, and NO^-. The amount of nitric oxide released from *S*-nitrosothiols has been determined by the rate of hemoglobin oxidation to methemoglobin. The high rate of transnitrosation has been attributed to the ability of nitrosothiols to act as NO^+ donors, the rate of NO^+ formation being higher than that of NO release. To study the NO^- release, the following model system has been used:



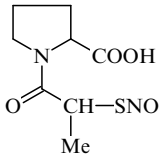
$$HNO + RSH \longrightarrow RSNHOH \xrightarrow{RSH} RSSR + NH_2OH,$$

$$NO^- + 2NO \longrightarrow NO_2^- + N_2O,$$

$$2NO^- + 2H^+ \longrightarrow N_2O + H_2O.$$

The fast formation of disulfides is accompanied by the release of hydroxylamine and N_2O, which is indicative of NO^- release. A conclusion is drawn from the experimental data that under physiological conditions *S*-nitrosothiols can act as NO^+, NO, and NO^- donors. The authors advocate that the activity of these compounds is due not only to homolytic but also to heterolytic cleavage of the S—N bond. The papers dedicated to the synthesis of new, and analysis of the already known *S*-nitrosothiols are rather numerous.[55,87–92] Special mention should be made of the synthesis of *S*-nitrosocaptopril **41**.[88] This compound, which structurally represents a hybrid molecule, can influence both vital systems that maintain arterial pressure in living organisms

and can induce vasodilatation both through activation of SGC and inhibition of the angiotensin-converting enzyme (ACE).[93]

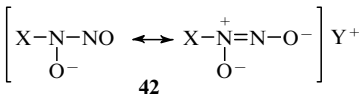


41

Indeed, it has been shown experimentally that compound **41** possesses vasodilating properties and inhibits ACE and platelet aggregation. According to the authors, compound **41** can be used in the treatment of hypertension and various cardiovascular diseases; however, in our opinion, the clinical application of this compound is hardly possible because of its low stability.

5. NO Complexes with nucleophiles

One more group of compounds that can act as nitric oxide donors is represented by the so-called nonoates **42**, in which the ON—NO dimer is formally bound to the nucleophile through the nitrogen atom.[94,95]



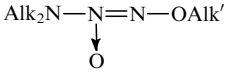
42

X = Et_2N, Pr^iNH;
Y = Na, NH_4, $RR'NH_2$.

These compounds are very stable as such, however, in solutions at pH 7.4 they are readily hydrolysed to give nitric oxide. In this case, like nitrosothiols, they do not require either reducing or oxidising agents for their hydrolysis. The rate of degradation is determined by the structure of the compounds and varies within a very broad range. Biological assays of this type of compounds have revealed that they are active vasodilating agents, platelet aggregation inhibitors, and regulators of cytochrome P-450 activity.[39,96,97]

To localise the NO release in definite organs and tissues, the above system has been incorporated into a polymeric matrix. Various types of polymers have been synthesised, *e.g.*, by incorporation of $Et_2N[N(O)NO]^-Na^+$ into polyethylene glycol, fusion of $H_2NCH_2CH_2N[N(O)NO^-]CH_2CH_2NH_3^+$ with polycaprolactam, and polymerisation of $H_2N(CH_2)_3N.[N(O)NO^-](CH_2)_3NH_3^+$ with dextran.[39] It has been established that the polymers containing the $[N(O)NO]^-$ fragment and stored *in vivo* as localised sources of nitric oxide can slowly release NO at pH 7.0 and 30 °C. These polymers prevent platelet aggregation and inhibit cell proliferation *in vitro*.

Another pathway of NO release in the vicinity of a biological target is the creation of prodrugs incapable of releasing nitric oxide without preliminary metabolic transformation into the nonoate by enzymes specific for this particular cell target. To this end, the following alkyl derivatives have been synthesised:

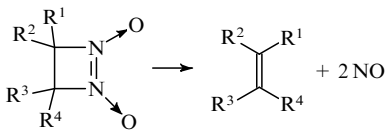


These compounds can release nitric oxide in solution and exhibit hypotensive activity *in vivo*.[98]

6. Heterocyclic derivatives

a. 1,2-Diazete-1,2-dioxides

This class of compounds has been studied relatively recently.[41,99,100] Compounds of the **43** type are known to decompose in aqueous solutions on moderate heating at physiological pH values to give nitric oxide. These data have been obtained using polarography[41] and EPR spectroscopy.[100]



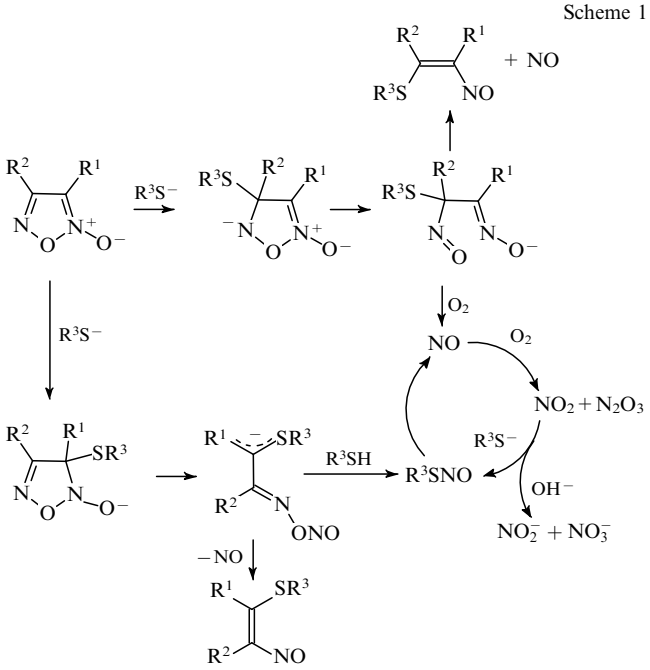
43

Electrochemical studies of a series of 1,2-diazete-1,2-dioxides **43** have revealed that the rate of NO release depends on the structure of the radicals and varies within a rather broad range. Compounds **43** are potent activators of SGC, manifest a noticeable spasmolytic effect *in vitro,* and effectively lower arterial pressure *in vivo*. In all cases studied (rate of NO generation, SGC activation, as well as spasmolytic and antihypertensive effects) there was a convincing correlation between the experimentally determined series of activities. The rate of NO formation is the determining factor in the manifestation of the above biological effects. The highest activity was observed with compound **43** [R^1 = Br, R^2–R^3 = $(CH_2)_4$, R^4 = Me] and the lowest activity, with compound **43** [R^1 = R^4 = H, R^2–R^3 = $(CH_2)_5$].

b. Furoxanes

A number of investigations have been dedicated to the study of the NO donor properties of furoxanes. It has been shown[101] that the release of nitric oxide takes place in the reaction of furoxanes with thiols (Scheme 1).

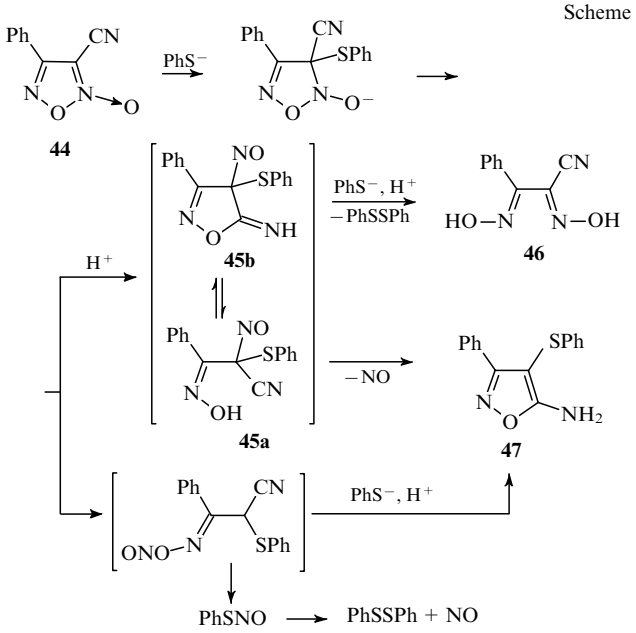
Scheme 1



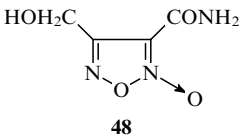
Furoxanes activate SGC and enhance the coronary blood flow. According to Feelisch *et al.*,[101] furoxanes can be regarded as prodrugs that exert their action *via* the NO/cGMP pathway. The thiol-dependent release of nitric oxide from furoxanes has been studied in detail with the interaction of 4-phenyl-furoxane-3-carboxynitrile **44** with cysteine and thiophenol as an example. Compound **44** activates SGC, produces strong vasodilating action, and demonstrates high antiaggregating activity. The yield of NO_2^- formed upon oxidation of NO in the presence of 5 mmoles of cysteine is 14±2% (after 1 min), 29±1% (after 5 min), and 40±2% (after 1 hour), and in the presence of 0.5 mmoles of thiophenol it is 64±2% within 1 hour. The reaction of **44** with thiophenol has been studied in detail[102] and is summarised in Scheme 2.

Compound **46** has been isolated in 20% yield, whereas isoxazole **47** formed from the intermediates **45a** ⇌ **45b**, in 60% yield. The experimental data provide an idea about the plausible

Scheme 2

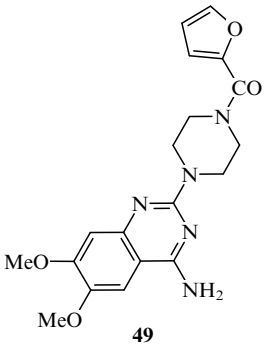


scheme of furoxane transformations leading to NO. A large series of biologically active furoxanes have been synthesised.[103–105] No tolerance to 4-hydroxymethylfuroxane-3-carboxamide **48** has been noted.[104]

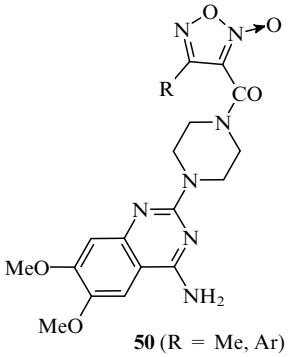


48

In contrast with the above findings, Hecker *et al.*[105] have shown that the release of nitric oxide is not thiol-dependent. The lack of tolerance makes it possible to use furoxane derivatives in the treatment of cardiovascular diseases.[105] One more study [106] is dedicated to the synthesis of hybrid molecules, furoxane analogues of the known drug prazosin **49**, in which the furyl fragment has been substituted by the furoxanyl one (compound **50**).

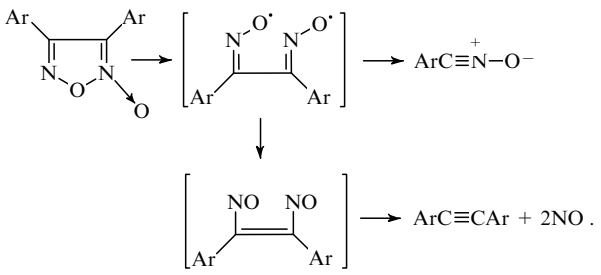


49



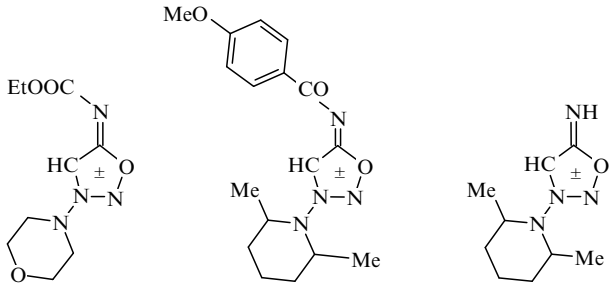
50 (R = Me, Ar)

Compounds **50** act primarily as antagonists of α_1-adrenoreceptors. Although their action is weaker than that of prazosin. However, at higher concentrations the ability of these compounds to release NO also has a part in the vasodilating effect. And, finally, worth noting is the study,[107] in which the cleavage of the furoxane ring upon electron impact mass spectrometry has been studied. The proposed fragmentation rationalises the formation of nitric oxide:



c. Sydnonimines

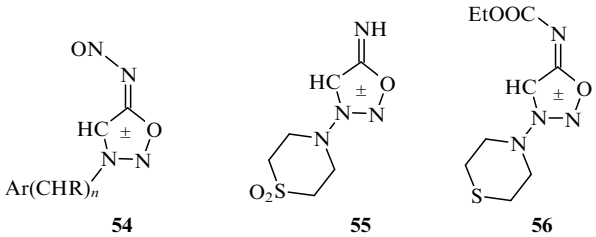
Another important class of heterocycles, for which the release of nitric oxide is characteristic under certain conditions, is represented by sydnonimines. Molsidomine **51**, one of the most potent antihypertensive drugs, belongs to this series.[108] Data on the high activity of this mesoionic heterocycle appear regularly in current literature (see, *e.g.*, Refs 55, 92, 109–113). Among compounds of this class, special mention should be made of the highly active nitric oxide donor, pirsidomine **52**, the biological activity of which is similar to that of isosorbide mononitrate, and the major metabolite of pyrsidomine **53**.[109]



51 **52** **53**

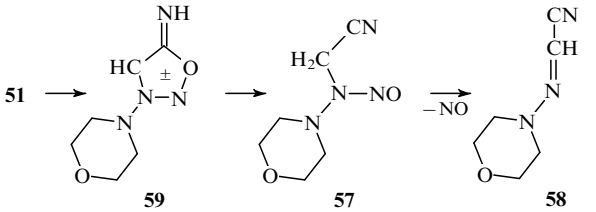
Unlike isosorbide mononitrate, compounds **52** and **53** do not induce tolerance, which is consistent with the hypothesis on the difference in the action mechanisms of sydnonimines and nitrates. In contrast to nitrates and, presumably, furoxanes, the generation of NO by sydnonimines does not require thiols.[109]

The high antithrombotic activity has been observed for sydnonimines with different substituents, *viz.*, *N-exo*-nitroso derivatives **54** [110] and compounds having thiazine residues at position 3 **55**, **56**.[111,113] It is noteworthy that the activity of compound **56** is somewhat lower than that of its morpholine analogue, molsidomine **51**.[113]



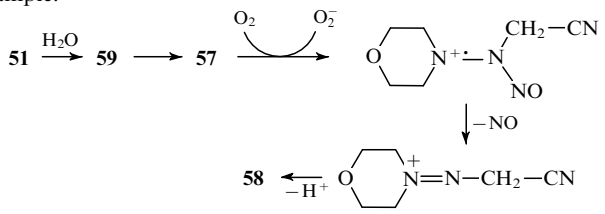
54 **55** **56**

It is stated [112] that sydnonimines are prodrugs and that their antianginal effects are due to the release of nitrogen oxide: the first stage of formation of type **57** metabolites from molsidomine occurs fast in neutral media. The formation of compound **58** and NO is accompanied by oxygen uptake.



59 **57** **58**

On the whole, the action mechanism of sydnonimines is directly coupled with two processes, *viz*., hydrolysis and oxidation, and can be depicted as follows with molsidomine **51** as an example:[9]



Thus, the main therapeutic effects of sydnonimines are their vasodilating and antiaggregating actions caused by the release of nitric oxide. Sydnonimines induce the activation of SGC, which is characteristic of all NO donors; however, their conversions do not require the presence of thiols. Significantly, the manifestation of biological activity of sydnonimines is accompanied by oxygen uptake. A close correlation has been found between the O_2 uptake and NO release (correlation coefficient, $r = 0.913$).[114] The decomposition of sydnonimines generates the superoxide anion (O_2^-); the relative rates of oxygen uptake and O_2^- generation correlate. The decomposition of sydnonimine always results in the formation of nitrites and nitrates.[114]

$$2NO + O_2 \longrightarrow 2NO_2,$$

$$2NO_2 \rightleftharpoons N_2O_4,$$

$$NO_2 + NO \rightleftharpoons N_2O_3,$$

$$N_2O_3 + H_2O \longrightarrow 2NO_2^- + 2H^+,$$

$$N_2O_4 + H_2O \longrightarrow NO_2^- + NO_3^- + 2H^+.$$

In addition, the presence of a superoxide anion in the medium can give rise to side processes.

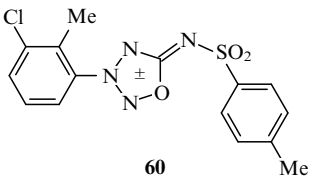
$$2O_2^- + 2H^+ \rightleftharpoons H_2O_2 + O_2,$$

$$NO + O_2^- \longrightarrow ONOO^- \overset{H^+}{\rightleftharpoons} ONOOH \rightleftharpoons \dot{N}O_2 + \dot{O}H.$$

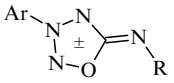
It is speculated [114] that the formation of not only NO, but also of O_2^- is essential for the manifestation of the therapeutic effect of sydnonimines, because the superoxide anion is rather active and previously considered to be the endothelium-derived contracting factor (EDCF, *cf*. EDRF, see above). A comparative study of 3-morpholinosydnonimine **59** and some other NO donors has been carried out.[92] Thus compounds **59** and *S*-nitroso-*N*-acetylcysteine (the so-called direct nitric oxide donors) release NO which is actively consumed by thiols in the following order: *N*-acetylcysteine > cysteine > glutathione. According to this classification, indirect NO donors (nitrates, molsidomine) do not decrease the number of SH-groups without pretreatment with the xanthine/xanthine oxidase system. Interestingly, sodium nitroprusside is also referred to as indirect NO donors, since at low concentrations it hardly decreases the number of SH-groups in solution, and its behaviour more resembles that of nitroglycerin than that of **59** or nitrosothiols.[92]

d. Other heterocyclic compounds

Derivatives of some other mesoionic heterocycles also seem to act following the pattern seen in sydnonimines. Thus it has been shown that a novel nitric oxide donor, the oxatriazole derivative **60**[115] possesses high vasodilating activity and, furthermore, enhances the effects of some other NO generators, *e.g*., sodium nitroprusside.



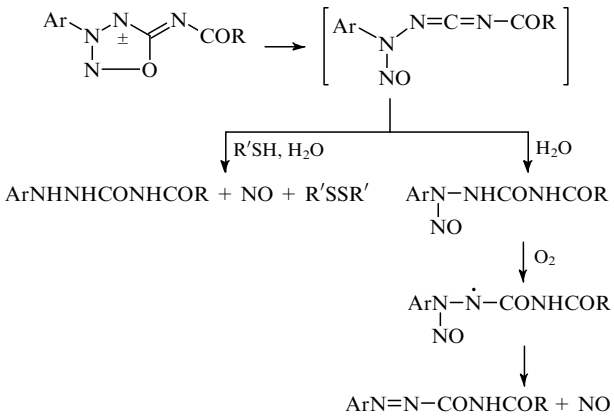
60



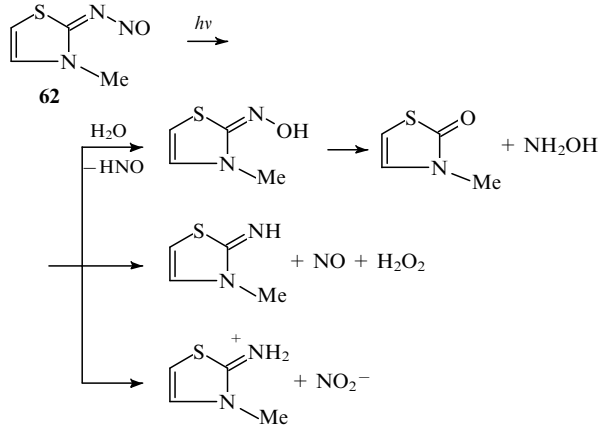
61

Related compounds of the general formula **61** manifested high cytotoxic and cytostatic activity. Taking into account the vascular effect of the above compounds, it may be assumed that they are promising tools in the treatment of tumours when combined with radiation and chemotherapy.[115]

Some other derivatives of oxatriazole also manifest antiaggregative properties.[116] It is assumed that their NO-generating capacity is due to the ring-opening. A series of *N*-nitrosohydrazines, *N*-nitrosohydrazides, and *N*-nitrosohydrazones have been synthesised,[116] they have also turned out to be platelet aggregation inhibitors *in vitro*.[116] According to Karup *et al*.,[117] these mesoionic systems release NO *via* the following hypothetical routes:



Not only mesoionic heterocycles but also *N*-nitroso derivatives of amino heterocycles, such as thiazole-2-nitrosoimines **62**, can be the source of NO.[118]

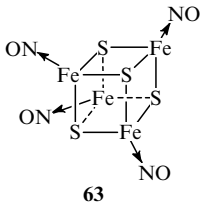


In the presence of glutathione, the corresponding *S*-nitroso derivative is formed. Upon irradiation, compound **62** activates SGC *in vitro*. A prerequisite for this activation is the presence of thiols.[118]

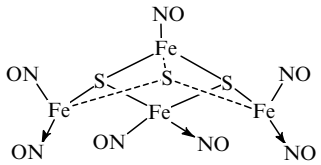
Among other derivatives of heterocycles capable of releasing nitric oxide are: pyridyl-1,2,5-oxadiazolecarboxamide 2-oxides,[119] derivatives of oxadiazolopyrimidine *N*-oxides,[120] and benzo-1,2,3-triazine *N*-oxides [121] that are used as cardiovascular agents.

It is interesting to note that some antitumour drugs, such as the alkaloids vincristine, vinblastine, and colchicine, as well as flavonoacetic acid (5,6-dimethylxanthenone 4-acetic acid) are potent activators of NO-S (the mechanism of this activation is presently uknown) and thus manifest 'indirect' NO-donor properties.[122]

And, finally, a novel class of NO generators possessing vasodilating properties is represented by Fe–S-nitroso clusters **63** and **64**.[123]



63

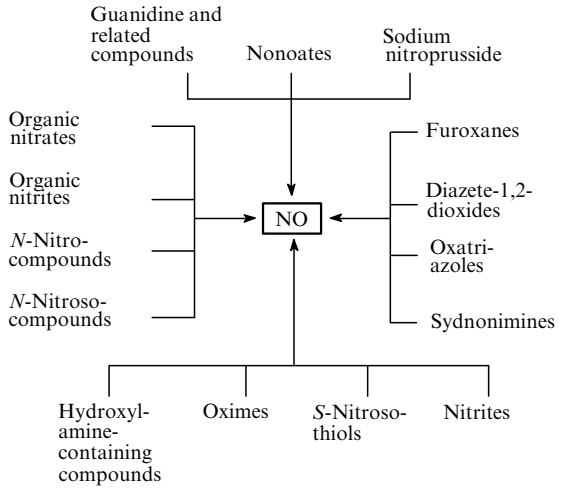


64

As expected, in the presence of oxyhemoglobin (the NO 'trap') or Methylene Blue (the SGC inhibitor) the biological activity of **63** and **64** drops drastically.

Most of the data presented herein concerning exogenous nitric oxide donors can be summarised in the Scheme 3.

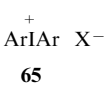
Scheme 3



This scheme illustrates the possibilities of control of the nitric oxide level in the organism through the introduction of NO generators having specific action mechanisms. Another approach to the regulation of the nitric oxide level in organs and tissues is the inhibition of NO-S responsible for NO production.

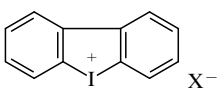
IV. Inhibitors of NO-synthase

The foregoing sections dealt with the ways of the increasing the level of nitric oxide in organs and tissues under conditions of its insufficient production in living organisms. However, a number of pathological states, including cardiovascular diseases, infections, brain injuries, *etc*., are accompanied by excessive release of NO. The undesirable toxicological effects of NO overproduction include extremal hypotension and/or cardiovascular collapse, cell injuries, and septic shock.[10] Therefore, in some cases the necessity to inhibit NO-S has been mentioned. As above, the synthesis of nitric oxide is performed by NO-S isoforms.[124] Both c-NO-S and i-NO-S produce nitric oxide through the oxidation of the guanidine fragment of L-arginine by utilising molecular oxygen and NADPH as a cosubstrate. All the isoforms contain FAD, FMN, and heme iron as prosthetic groups and require BH_4 as a cofactor. Correspondingly, flavoprotein inhibitors can suppress the activity of NO-S. Thus the iodonium salts **65** and **66** and ebselenium (**67**), the selenium analogue of isothiazole, are potent inhibitors of NO-S.[10]

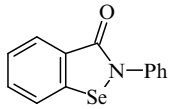


65

Ar = Ph, 2-thienyl

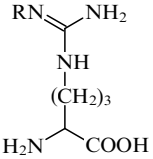


66



67

Arginine derivatives and related compounds are most commonly used as NO-S inhibitors. Since several reviews concerning approaches to the synthesis of NO-S inhibitors have been published recently,[4, 6, 10] here we shall dwell only on some of the most important and recent studies in this field. It should be mentioned, however, that the most important NO-S inhibitors are N^{ω}-methyl- **68** and N^{ω}-nitro-L-arginines **69**[10] whose properties are still the subject of most intensive studies.[125–129]



68: R = Me;

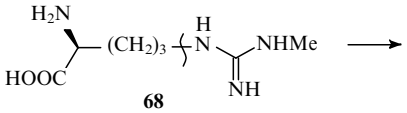
69: R = NO_2;

70: R = $CH_2=CH-CH_2-$;

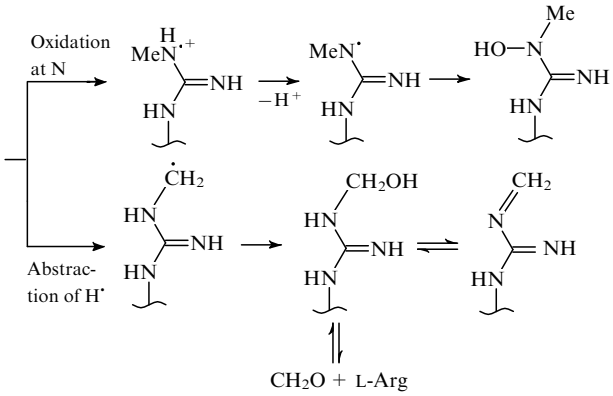
71: R =  .

68–71

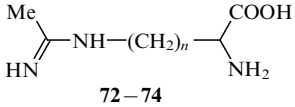
Thus compound **68**, the inhibitor of macrophage and i-NO-S, manifests its activity particularly through the alkylation of the enzyme.[125] It has been established that methylarginine **68**, similarly to aminoguanidine, inhibits NO-S and thus prevents the development of diabetic vascular functions.[126] Interestingly, methyl esters of the amino acids **68** and **69** manifest an analgesic effect that is ascribed to their influence on the central nervous system.[127] Inhibitors **68** and **69** potentiate the hypertensive effect of adrenaline.[128] Essentially, nitroarginine **69** induces strong arterial hypertension that can partly be prevented by administration of L-arginine, but not of D-arginine.[129] It was assumed [130] that compound **68**, being an irreversible inhibitor of macrophage i-NO-S, is subject to NO-S action according to the following two schemes:



68



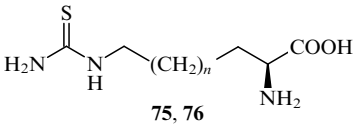
Olken and Marletta [131] and Feldman [132] have synthesised two novel i-NO-S inhibitors, *viz*., N^{ω}-allyl- **70** and N^{ω}-cyclopropyl-L-arginines **71**. It is interesting that whereas the former is an irreversible inhibitor, the latter is reversible, *i.e*., the effect of compound **70** is similar to that of nitroarginine **69**. The reasons for such a difference in the effect of compounds **70** and **71** are unclear. A series of structural analogues of arginine as NO-S inhibitors have been described.[21, 133–135] Thus i-NO-S inhibitors (substituted arginines and homoarginines) have been used in the treatment of systemic hypotension caused by pathological 'overproduction' of nitric oxide.[134] As i-NO-S inhibitors, N^6-(1-iminoethyl)-L-lysine **72**, N^5-(1-iminoethyl)-L-ornithine **73**, and N^6-(1-iminoethyl)-D,L-homolysine (**74**) have also been examined. It has been found [21] that compound **72** is a strong selective inhibitor of i-NO-S, whereas the ornithine **73**, which is also an active inhibitor of i-NO-S, does not possess any selectivity and affects c-NO-S with equal strength.



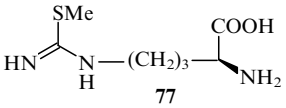
72: $n = 4$;
73: $n = 3$;
74: $n = 5$.

72–74

The synthesis of L-thiocitrulline **75**, L-homothiocitrulline **76**, and *S*-methylthiocitrulline **77** has been performed.[136] These analogues and derivatives of L-citrulline, the product of L-arginine oxidation in living organisms, are potent inhibitors of neuronal c-NO-S and i-NO-S. It is noteworthy that compound **77** is much more active in comparison with methylarginine **68**. The stereoselectivity of its interaction with the enzyme has been stressed again, and the activity of only L-enantiomers has been noted. D-isomers are fully devoid of the inhibitory activity.



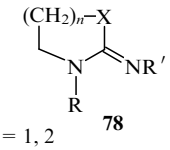
75, **76**



77

75: $n = 1$; **76**: $n = 2$.

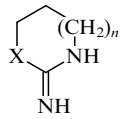
Compounds **75** and **77** are potent pressor agents *in vivo*. The basic idea of the synthesis of this novel class of inhibitors was that the presence of a sulfur atom might result in their strong binding to the active centre of the enzyme in the vicinity of the heme iron, *i.e.*, this might enhance the interaction of the inhibitor with the enzyme. The experimental data seem to confirm this hypothesis.[136] One of the molecular fragments of arginine and related compounds responsible for the binding to NO-S is, undoubtedly, the guanidine moiety. Clearly, simple derivatives of guanidine and various amidines have also been tested as NO-S inhibitors.[137–142] It has been shown that aminoguanidine is an NO-S inhibitor,[126,137] like N-arylguanidines[138] and cyclic guanidines or amidines of the general structure **78**.[139]



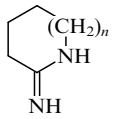
78

$n = 1, 2$

Compounds **78** preferentially inhibit i-NO-S.[139] Isothioureas and cyclic guanidines of the type **79**, cyclic amidines (**80**), and related compounds[140–142] are active inhibitors of NO-S.



79

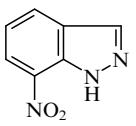


80

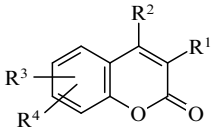
X = O, S, NH; $n = 0–4$.

Some of the above-mentioned compounds can inhibit i-NO-S *in vivo*. Despite the great number of novel NO-S inhibitors having guanidine and amidine groups, the amino acid inhibitors **68**, **69**, and **73** are still most frequently used.[143–146]

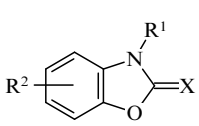
Novel inhibitors have been detected in the series of various heterocycles. They include 7-nitroindazole **81**,[147–149] coumarin derivatives **82**,[150] and benzoxazole derivatives **83**.[151] Compound **81**, possessing high analgesic activity, may be used as the 'starting material' in the elaboration of selective NO-S inhibitors acting on the CNS without any cardiovascular effect.



81



82

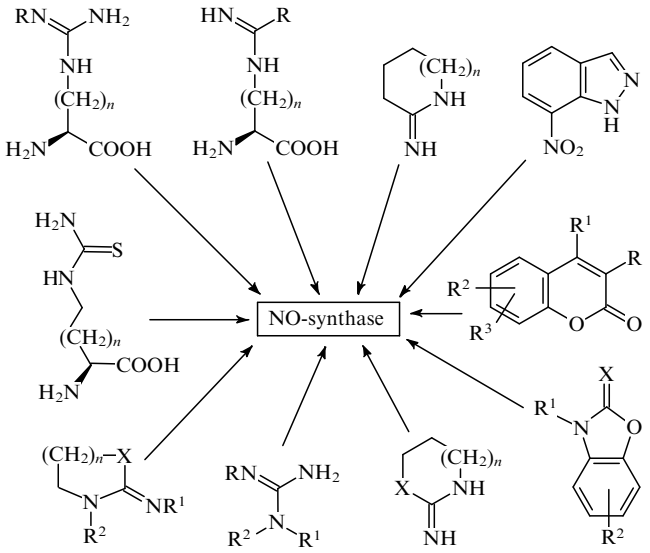


83

The data on inhibition of NO-S by compounds of various types considered in the above sections have been summarised in Scheme 4.

For the sake of completeness, it is necessary to stress once again that iron-heme-containing proteins and, correspondingly, heme-binding agents are inhibitors of NO-S. Thus carbon

Scheme 4



monoxide is a good ligand for Fe^{2+} and a potent inhibitor of NO-S. Other heme-ligands of the cyanide type and even nitric oxide itself can play the role of inhibitors.[10,152]

V. Conclusion

Despite the fact that the enzymic formation of nitric oxide in vascular endothelial cells was discovered only in 1987, it follows from the above data that this biological mediator has aroused considerable interest among investigators, and the number of publications in this particular field of chemistry, biology, and medicine is steadily increasing. It can be emphasised once again that the present review deals primarily with the chemical and structural problems connected with the possibility of creating novel types of compounds able to generate or, contrariwise, inhibit NO production in the organism. Hence, a vast number of purely medical and biological problems, which are irrelevant to the subject of our discussion, have fallen outside the scope of this review. Also, the review gives relatively little information about such important problems as nitrate therapy; little attention is given to the well-known drug, sodium nitroprusside. Biological and clinical applications of the drug molsidomine have been described in less detail than they deserve. Problems of carcinogenicity and mutagenicity of *N*-nitroso compounds (*e.g.*, *N*-nitroso-*N*-methylurea and related compounds, such as *N*-methyl-*N*-nitroso-*N'*-nitroguanidine) have been described in only little detail. These problems have been considered in a number of the above-cited and other reviews[1,153–155] and the references cited therein.

In the contemplation of the above data, quite a large number of problems still remain unsolved, and even experimental data are often at variance with one another. This refers to differences in the pathways of chemical and enzymic oxidation of guanidine fragments; to the problems pertaining to the tolerance of nitrates that can be thiol-dependent or independent of the content of thiol compounds; to the demand for thiols upon NO release from furoxanes (for reference, see one of the most recent publications[156] in which it has been shown that some furoxanes can release NO in the absence of thiols, whereas for structurally related furoxane derivatives thiols are necessary); to the simultaneous release of nitric oxide and the superoxide anion; to the 'indirect' NO-generating effect of sodium nitroprusside, and so on.

Unquestionably, it is important to establish (although it is currently difficult to evaluate), to what extent the high reactivity of nitric oxide can influence some particular biochemical processes. For example, it has recently been established that NO oxidises dihydropyridines to pyridines.[157] When dihydropyridine

systems in the organism, such as NADH and NADPH, are considered, it is clear that this phenomenon needs special analysis.

After this review had been prepared for publication, a paper was published [158] that may have a great impact on the approach to problems related to NO formation from arginine and NO donors. It describes the discovery of 1*H*-[1,2,4]-oxadiazolo[4,3-*a*]quinoxalinone-1 (SGC), which is the first direct inhibitor of NO-induced activation of SGC. The use of this compound presents indisputable interest, although it is still unclear what new avenues in the understanding of the role of NO it will open.

All these problems still await their solution. However, even now it can be predicted with certainty that the efforts aimed at creating novel generators of nitric oxide and specific inhibitors of NO-S isoforms hold much promise and represent a perspective trend in the search for novel effective drugs that has already turned out to be highly profitable in the theoretical sense. Further developments in this field may culminate in impressive achievements, including practical ones, even in the nearest future.

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