Reviews

Nitric oxide synthase inhibitors: biology and chemistry

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The review is devoted to the problems related to inhibition of the generation of nitric oxide, a versatile regulator of cell metabolism, whose excessive production is responsible for various pathologies. The approaches to the preparation of inhibitors are discussed and the prospects for the synthesis of inhibitors selective with respect to various NO-synthase isoforms are considered; these aspects largely determine the possibility of using these compounds in medicine. The structures of some classes of inhibitors are presented and their biological properties and the main applications for arresting pathological states are discussed.

Key words: nitric oxide, nitric oxide release, NO-synthases, NO-synthase inhibitors, soluble guanylate cyclase, agonists, antagonists, inhibitors, pharmacology, drugs.

1. General

Due to the enormous interest in the biological role of nitric oxide, a substantial number of new studies concerning NO donors and NO synthase (NOS) inhibitors appeared during the five years elapsed after the publication of a review.[1] The present review covers only the most important recent studies. They demonstrate the significance of this problem for medicine and biology and the diversity of possible chemical approaches to the synthesis and elucidation of structural features of systems whose investigation can ensure the progress in this field. There are two aspects of the problem of NO release in the organisms of mammals: both a deficiency and an excess of NO in the organism result in pathological states.

Our previous review[2] devoted to compounds able to generate nitric oxide under various conditions, in particular, *in vivo*, covers only the former aspect of the problem. The deficiency of NO brings about a number of severe consequences* because NO, though being a cytotoxic and cytostatic agent, plays nevertheless a key role in the vascular tone control and participates in the maintenance of homeostasis,** in breath and immunity regula-

* Cardiovascular, infectious, and inflammatory diseases, thromboses, malignant tumors, diseases of urogenital system, cerebral damages caused by strokes, *etc*.

** A physiological process in which the key parameters of the internal environment (blood pressure, body temperature, acid-base balance, *etc*.) are maintained at equilibrium despite changes in the environmental conditions.

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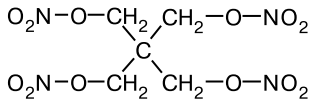
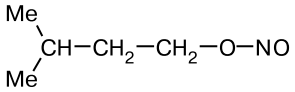
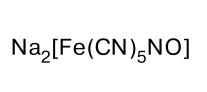
tion, and in neurotransmission mechanisms. Therefore, the quest for compounds capable of generating nitric oxide in the organism is now a vigorously developing line of research. The activity of a number of drugs currently used in medical practice is attributed with sufficient reasoning to their ability to release nitric oxide *in vivo*. These are drugs such as nitroglycerin, isosorbide dinitrate, isosorbide mononitrate, pentaerythritol tetranitrate, amyl nitrite, *i.e.*, antianginal drugs* (Latin *angina pectoris* means breast pain) belonging to the group of peripheral vasodilators (vasorelaxant remedies). Some other well-known NO donors such as sodium nitroprusside and molsidomine are also attributed to this group.



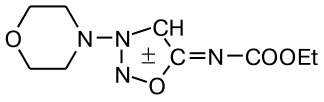
Pentaerythritol tetranitrate



Amyl nitrite



Sodium nitroprusside



Molsidomine

However, accumulation of excess nitric oxide in the organism is an equally important problem. Since nitric oxide is able to penetrate virtually any biological membrane by diffusion, its excessive release may cause a series of various, often serious, pathological states. These include septic shock,** neurodegenerative diseases, and inflammatory processes. The generation of endogenous NO is known to be due to L-arginine oxidation by NO synthases (NOS). To avoid excessive production of this compound, the use of NOS inhibitors is required. This review is devoted to the structural features and biological significance of these inhibitors.

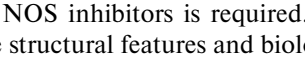
At present, the key problem in the search for inhibitors of nitric oxide synthesis is related to the fact that the endogenous production of NO may be performed by a whole family of NOS and the distribution and properties of these enzymes largely determine their functions. Thus, it is a paramount necessity to look for not merely NOS inhibitors but rather substances that act selectively on these enzymes.[3]

Three forms of NOS have now been identified. One of them is a constitutive neuronal isoform (nNOS or NOS I) found in the central and peripheral nerve tissue. The nNOS activity is regulated by Ca^{2+} and calmodulin.*

Yet another constitutive enzyme, eNOS or NOS III (which is also calmodulin/Ca^{2+}-dependent), has been found in vascular endothelial cells. The third, Ca^{2+}-independent enzyme (iNOS or NOS II) is an inducible form that appears in response to, for example, inflammation or bacterial infection.

Table 1 summarizes some characteristics of NOS,[4] namely, localization in organs and tissues and ways of activation of each isoform in the organism.

The difference between the isoforms is determined by the fact that the constitutive enzymes (eNOS and nNOS) are permanent cell and tissue constituents present in really measurable amounts, whereas iNOS is apparently virtually missing from most of cells until its expression is induced by endotoxins (lipopolysaccharides, LPS) and/or various cytokines such as interferon-γ, interleukin-I, the tumor necrosis factor-α.** These and other cytokines provoking inflammation are bound to macrophages and trigger a sequence of processes leading to iNOS synthesis. After the synthesis, iNOS diffuses to a damaged, *e.g.*, tumor cell located near the macrophage. There, the resulting NO interferes in a series of processes taking place in the rapidly growing cell. A key target of NO action is inhibition of aconitase, the enzyme catalyzing an early step of the citric acid cycle (citrate to isocitrate transformation). The resulting nitric oxide also interferes in the electron transport chain. In addition, NO inhibits ribonucleotide reductase thus blocking the DNA biosynthesis in the tumor cell. The pathophysiological effects of nitric oxide are related not only to its direct action on various structures of organisms but also to its reactions with other molecules, for example, with the superoxide radical anion, resulting in the peroxynitrite anion:



The synthesis of NO by macrophages is a response of the immune system to an infection or tumorigenesis. In addition to nitric oxide, macrophages produce a substantial amount of superoxide, the rate of this reaction to give peroxynitrite being controlled by diffusion. Protonation of the peroxynitrite anion yields the corresponding acid,

* Drugs increasing the blood supply of heart or decreasing its oxygen demand (prevention or relief of stenocardia attacks).

** An acute life-threatening pathological process associated with the formation of suppurative inflammation foci in organs and tissues.

* Calmodulin is a protein acting as an intercellular mediator of calcium transfer.

** Cytokines are soluble factors produced by cells for biological action on the nearest cells.; interferons are substances produced by cells when acted upon by viruses, which are able to suppress their growth; interleukins are proteins that control the formation of blood cells and platelets and the immune reactions; tumor necrosis factor α is a specific protein inducing destruction of malignant cells.

Table 1. Nitric oxide synthase (NOS) isoforms

NOS type (monomer molecular mass, kDa)	Alternative names	Distribution among tissues and cells	Activation type
NOS-I (155)	Neuronal NOS (nNOS); brain NOS; type I NOS	Neurons of the central and peripheral nervous systems, uterus, skeletal muscles	Constitutive form, calcium/calmodulin-dependent
NOS-II (125)	Inducible NOS (iNOS); type II NOS	Macrophages, liver, unstriped muscles, endothelium, heart	Induced by lipopolysaccharides (LPS), cytokines, and glucocorticoids, calcium/calmodulin-independent
NOS-III (133)	Endothelial NOS (eNOS); type III NOS	Endothelium, heart, brain	Constitutive form, calcium/calmodulin-dependent

which further decomposes to give nitrogen dioxide and hydroxyl radical, which is a very strong oxidant and a cytotoxic agent. Thus, the peroxynitrite and the hydroxyl radical, highly reactive with respect to cell components, contribute to the total cytotoxic effect of the nitric oxide released by macrophages.[5]

The pathological states that arise due to excessive nitric oxide production by NO synthase isoforms are characterized in Table 2.

Now we consider the most expedient routes for the search for NO synthase inhibitors.[3]

1. The search for compounds able preventing the delivery of L-arginine to the enzyme active site. This approach is used most often to prepare the inhibitors.

2. The synthesis of agents inactivating the cofactors needed for the NOS-catalyzed oxidation of L-arginine. In this case, the most attractive and practicable routes include the search for substances decreasing the level of Ca^{2+} in cytosol,* inhibitors of tetrahydrobiopterin (BH_4) synthesis, and calmodulin antagonists.

3. The search for inhibitors of the electron transport involving NADPH and flavins and also for agents interfering in the heme functions.

4. The search for NOS expression inhibitors.

5. The preparation of substances preventing the substrate (L-arginine) binding to the enzyme.

6. The synthesis of compounds that destroy the nitric oxide formed.

Analysis of the published data[2,6—19] shows that groups of compounds that compete with the substrate binding to the enzyme have been studied most extensively; first of all, we dwell on this type of system.

Table 2. Pathological states arising upon excessive nitric oxide production by various NO synthases

NO synthase	Pathology
eNOS	Early stages of inflammation, induction of vasodilation (blood vessel expansion), decrease in blood pressure
nNOS	Cerebral ischemia (exsanguination due to functional (spasm) or organic contraction or closure of the feed vessel), other ischemic diseases, neurodegenerative diseases including Parkinson´s and Alzheimer´s diseases, strokes, attacks, pain syndromes, neurotoxicity, psoriasis
iNOS	Multiple sclerosis, Parkinson´s and Alzheimer´s diseases and Hangtinton´s disease (hereditary nervous system disease), chronic inflammations, psoriasis, diabetes, septic shock, chronic arthrites, extreme hypotension, cardiovascular collapse, circulatory (blood circulation disturbance-induced) shock, and tumors including malignant ones

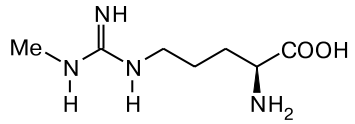
2. L-Arginine derivatives as NOS inhibitors

The endogenous NOS substrate, L-arginine, was found[20] to be itself an inhibitor of the constitutive eNOS when present in a concentration of >100 μmol L^{-1} (K_m of arginine as a substrate for NOS equals 3—5 μmol L^{-1}). The eNOS-inhibiting activity is inherent in none of the following species: D-arginine, NOS cofactors, nitric oxide binding compounds, oxyhemoglobin, enzymes (catalase or superoxide dismutase (SOD)), superoxide radical anion, hydrogen peroxide (compounds that accompany the enzymatic formation of nitric oxide in one or another way), or the products of enzymatic oxidation of L-arginine (*N*-hydroxy-L-arginine and L-citrulline). Apparently, eNOS contains a second site having tropicity to the substrate and binding to this site sterically inhibits the interaction with the active site responsible for L-arginine oxidation.[20] This precludes binding to the enzyme active site and results in inhibition of the catalytic activity.

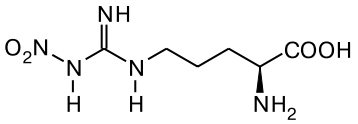
Here we consider a series of best-known NOS inhibitors and new data on their complexation with the active sites of enzymes and the degree of their selectivity with

* The aqueous phase of the cytoplasm (the cell contents surrounding the nucleus) with dissolved substances.

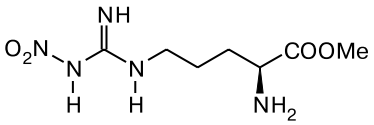
respect to a particular isoform. The use of structurally similar arginine analogs appears more attractive than the use of other agents because the close similarity to the substrate enables interaction of these analogs and arginine with the same cell enzymes. In this case, competition between the structurally similar compounds (the substrate and the inhibitor) for the enzyme active site is an important inhibition mechanism. From this standpoint, mention should be made of studies dealing with N^G-monomethyl-L-arginine (L-NMMA), N^G-nitro-L-arginine (L-NNA), and its methyl ester (L-NAME).



L-NMMA



L-NNA



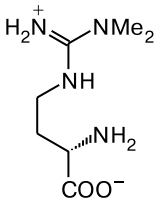
L-NAME

L-NAME is a prodrug; it is hydrolyzed in the organism to give the active inhibitor, L-NNA. This transformation requires involvement of esterases. The approach based on preliminary esterification followed by *in vivo* transformation of the ester into the acid allows deliberate tackling of the tissue selectivity problem, because in cases like L-NAME, inhibition can take place only in the tissues that contain specific esterases.[21] The mechanisms of NOS inhibition by various substituted guanidines and, in particular, arginine derivatives, are quite diverse. For example, L-NMMA is metabolized under the action of NOS being converted into *N*-hydroxy-*N*-methyl-L-arginine, which irreversibly inactivates the enzyme. Moreover, the enzyme loses the heme under the action of L-NMMA. However, in some tissues and cell types, the L-NMMA metabolism can afford L-arginine itself and, hence, L-NMMA can be used as an alternative substrate for the production of nitric oxide.[22,23]

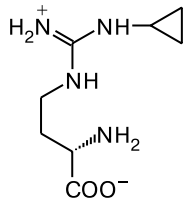
The oxidative transformations of L-NMMA are presented in Scheme 1.*

The oxidation of L-NMMA involving NADPH gives rise to NO, citrulline, formaldehyde, *N*-hydroxy-*N*-methyl-L-arginine, and L-arginine. It was shown that a number of processes (not all of them are shown in Scheme 1) afford large amounts of hydrogen peroxide. It is important that the above-noted destruction of the heme is not prevented by catalase, *i.e.*, does not depend on the H_2O_2 formation. Nevertheless, it is believed that NOS inactivation can largely depend on irreversible oxidative processes involving hydrogen peroxide and resulting in modification of amino acids or heme destruction.[22,23]

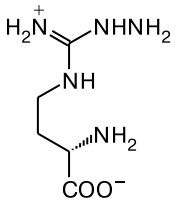
The nNOS inhibition by L-NNA starts as a process that can be suppressed by introducing an additional amount of arginine; however, this process becomes irreversible with time.[24] Conversely, the action of L-NNA on iNOS is reversible inhibition. An important conclusion has been drawn,[24] namely, that L-NMMA and L-NNA deactivate the enzyme by different mechanisms, although both are reversibly bound to NOS and compete with arginine. L-NMMA was identified as an antagonist slowly metabolizing to give NO and citrulline, while L-NNA is not metabolized by the neuronal enzyme. Presumably, L-NNA induces conformational changes in the enzyme active site, which accounts for the blockade of its oxidative activity toward L-arginine. Study of the kinetics of NOS inhibition by nitroarginine showed that inhibition has an irreversible component with respect to nNOS both *in vitro* and *in vivo*. In addition, it is stated in some publications that the view of nonselectivity of L-NNA is not exact because its action is clearly tissue-dependent; for example, inhibition of the bovine brain constitutive isoforms by L-NNA is much more efficient than that of the inducible enzyme. Detailed kinetic analysis of inhibition by nitroarginine demonstrated that, although the rate of its association with the enzyme is relatively low, the dissociation rate of the enzyme—inhibitor complex is so low that this complex can be isolated.[5] However, in general, N-substituted arginine derivatives exhibit low selectivity toward NOS isoforms and, having been introduced into isolated tissues and organs or *in vivo*, they block both the constitutive and inducible enzymes. This type of compound includes, apart from the substances mentioned above, a whole series of *N*-alkylarginines, for example, *N*,*N*-dimethylarginine, which is a strong inhibitor of all isoforms.



N,*N*-Dimethyl-L-arginine



N-Cyclopropyl-L-arginine

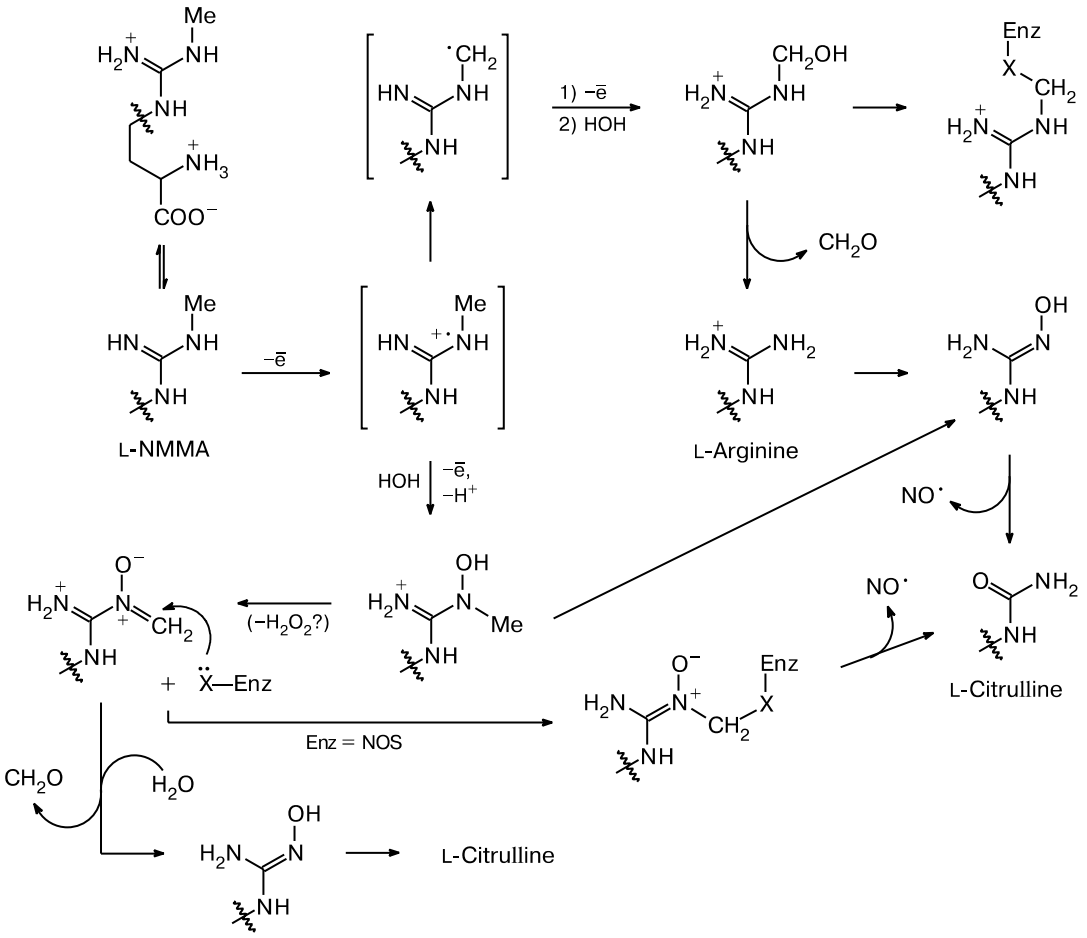


N-Amino-L-arginine

Nevertheless, selective inhibitors can also be found among this class of compound. Thus *N*-cyclopropyl-L-arginine acts *in vitro* on neuronal NO synthase approximately 400 times as strongly as on the inducible

* The given scheme is based on Refs 22, 23.

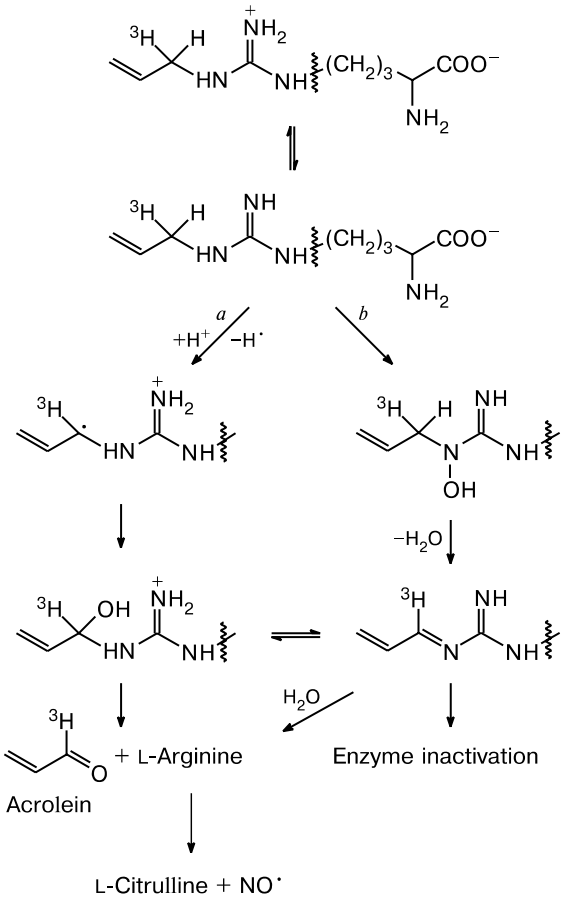
Scheme 1



NO-synthase. It is worth mentioning that both L-NNA and L-NAME inhibit preferably the constitutive forms rather than the inducible form, although the selectivity is moderate. A comparison of the efficiency of inhibition by various arginine analogs has been reviewed.[5] In the case of iNOS, the activity decreases in the following sequence of arginine derivatives: *N*-amino ≃ *N*-methyl (L-NMMA) ≫ ≫ *N*-nitro (L-NNA). However, a different order was found for constitutive NOS in the central nervous system tissues: *N*-nitro (L-NNA) ≫ *N*-amino ≃ *N*-methyl (L-NMMA). In the initial period of time, all "arginine antagonists" compete with arginine.[25] This indicates that they occupy the arginine/citrulline-binding active site of the enzymes. During this period, the degree of inhibition decreases upon the addition of arginine. As time goes by, the inhibition of NOS becomes irreversible (or slightly reversible). It is still unknown exactly what particular L-NMMA metabolites cause irreversible enzyme inactivation; no reliable data on the inhibition mechanisms by the other above-mentioned compounds of this type are available either. Presumably, L-NNA binds strongly to the enzyme, the resulting complex slightly dissociates and, thus, the substrate can no longer reach the active site.[26]

N^{ω}-Allyl-L-arginine and N^{ω}-cyclopropyl-L-arginine were first proposed as NOS inhibitors in 1992.[27] The detailed analysis of the putative mechanism of action of the former compound was published only recently.[28] N^{ω}-Allyl-L-arginine is a competitive inhibitor and irreversible inactivator for inducible and neuronal NO synthases. The inactivation is initiated by *N*-hydroxylation, which enables the reductive allylation of the heme coenzyme. N^{ω}-Allyl-L-arginine is not only an inhibitor but also an nNOS substrate. Labeled compounds, N^{ω}-[1-^{3}H]allyl-L-arginine and N^{ω}-allyl-L-[1-^{14}C]arginine, were used for elucidation of the mechanism.[28] The transformation of the inhibitor was shown to yield acrolein, water, and L-arginine. The routes to acrolein and water are described by the scheme presented below, which shows two alternative reaction pathways. One of them is based on the oxidation of the inhibitor to give a radical (pathway *a*) and the other includes *N*-hydroxylation (pathway *b*) followed by dehydration.

Scheme 2



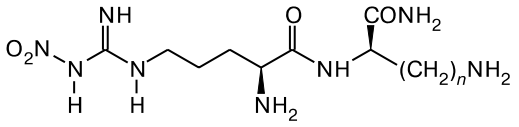
On the basis of kinetic measurements, it was concluded that oxidation involving carbon (pathway *a*) rather than nitrogen (pathway *b*) is the major route of transformation of *N*-allylarginine under the action of NO synthase, although the possibility of formation of *N*-hydroxy derivative through a minor reaction pathway is not ruled out. *N*-Allyl-L-arginine is a reversible inhibitor of the inducible isoform (K_i = 2.1 μmol L^{-1}); however, preincubation of this compound with iNOS and all necessary cofactors in the absence of L-arginine makes it a time- and concentration-dependent irreversible inhibitor of the enzyme with k_{inact} = 0.026 min^{-1} and K_i = 3.4 μmol L^{-1}. The *N*-cyclopropyl derivative is a reversible inhibitor with K_i = 7.7 μmol L^{-1}.

It was shown[29,30] that the nNOS and eNOS isoforms form nitric oxide owing to a slight increase in the intracellular concentration of calcium ions; iNOS (or the inducible form localized in macrophages* that was recently accepted for consideration by some researchers and designated mNOS) does not depend on the Ca^{2+} concentration. The main source of NO is normally skeletal muscles,[30] nitric oxide being largely responsible for muscle contraction and metabolic processes occurring in muscles. It was found that NOS inhibition increases the muscle strength and decreases muscle relaxation. Although NOS (mainly, eNOS and nNOS) were identified in skeletal muscles, the role of these isoforms in the muscle vital functions is not entirely clear yet. Chronic inhibition of the NOS activity in rats resulted in substantial loss of muscle bulk and substantial decrease in the motion activity.* [30] Study of the difference between the modes of action of L-NAME and L-NNA on the constitutive NOS (eNOS and nNOS) showed[31] that both compounds are active enzyme inhibitors but L-NNA is a direct inhibitor, while L-NAME requires preactivation. Using an isolated rat heart as a model, it was shown that the resulting effects of the two inhibitors do not differ quantitatively, the L-NNA effect being developed much faster (in 0.7 min) than that of L-NAME (4.2 min). The time of development of the L-NAME-induced effect is dictated by its transformation into nitroarginine (L-NNA). Freshly dissolved L-NAME is a 50 times less active nNOS inhibitor (IC_{50} = 70 μmol L^{-1}) than L-NNA (IC_{50} = 1.4 μmol L^{-1}) (IC_{50} is the inhibitor concentration that brings about a 50% decrease in the enzyme activity), but keeping in a neutral or alkaline solution makes their activities equal due to hydrolysis of the ester group.[21] These data indicate unequivocally that L-NAME is a prodrug inactive until converted into L-NNA. The bioactivation of L-NAME proceeds at a moderate rate in physiological buffer solutions (the half-life in a buffer solution with pH 7.4 is 365±11.2 min, that in a blood plasma is 207±1.7 min, and that in whole blood is 29±2.2 min), but it is sharply accelerated in tissues (for example, in vessel endothelia).

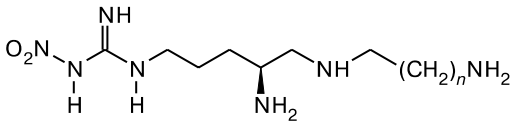
It has already been noted that the search for selective inhibitors of nitric oxide synthases is a fundamental problem for solving theoretical and practical problems of using these compounds in biology. The nNOS inhibition is quite important because excessive production of nitric oxide by this isoform located in neurons of the central nervous system leads to various neurodegenerative diseases such as Alzheimer´s disease, Parkinson´s disease, neuroimmune diseases and AIDS-accompanied demen-

* Large cells whose main functions include participation in the natural specific antitumor immunity. They are present in most tissues and organs.

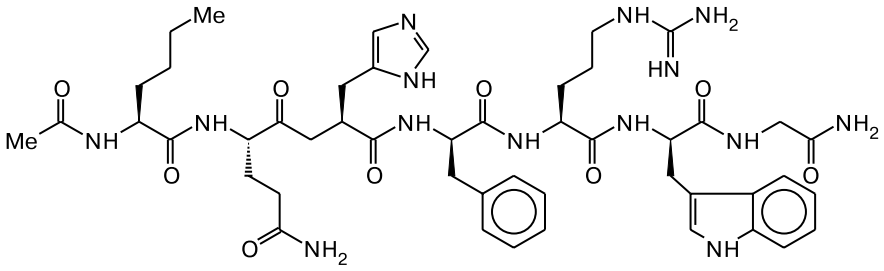
* An interpretation of the muscular atrophy and loss of motion (hypokinesia) following the introduction of L-NAME is based on the fact[30] that the inhibition of eNOS diminishes blood circulation in muscles and causes chronic ischemia and the loss of muscle function. An alternative (and more comprehensible, in our opinion) interpretation is based on the fact that nitric oxide is a signaling molecule included in the relaxation of skeletal muscles and needed for realization of their functional activity. The L-NAME-induced inhibition of the NO release decreases the NO concentration in tissues and, correspondingly, brings about the above-noted adverse physiological processes.



1, n = 1—3



2, n = 1—3



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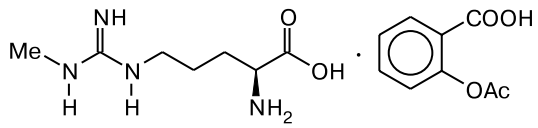
tia.[32—38] In a series of latest publications dealing with this topic,[39—41] attention has been paid to modification of L-NNA, *viz.*, to the synthesis of dipeptides containing a nitroarginine fragment (**1**) or their nonamide type analogs (**2**), exhibiting a very high selectivity with respect to nNOS.

Of compounds of type **1**, the homolog with $n = 1$ is the most active; it inhibits nNOS 1500 times more efficiently than eNOS and 192 times more efficiently than iNOS. The absence of amide substituents in the second group of compounds (**2**) accounts for their stability against enzymatic cleavage by peptidases. These compounds (n = 1—3) are also rather selective, they inhibit nNOS 2617, 1807, and 893 times more strongly than eNOS and 325, 252, and 267 times more strongly than iNOS, respectively.

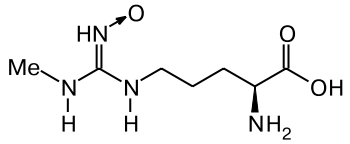
A fairly promising compound that is now under clinical trial as an antitumor agent, an analgesic, and an antiarthritis remedy is an analog of the heptapeptide α-melanocyte-stimulating hormone (**3**)* decreasing the production and inhibiting the action of a number of key cytokines such as interleukins-1β, -6, and -10 and the tumor necrosis factor TNF-α (see Section 1, Footnote on p. 1974) and iNOS production.[42a]

Of other compounds corresponding to the given type of inhibitors, we would like to mention the L-NMMA salt with acetylsalicylic acid, which is a nonselective inhibitor simultaneously for two important enzymes, *viz.*, cyclooxygenase (COX) and NOS.[43a]



This compound is recommended for the use in clinical practice (after completion of the clinical research) for treatment of cardiovascular and ischemic diseases (cerebral circulation abnormalities), as an antiinflammatory drug (for treatment of rheumatoid arthritis), and against immune diseases.

The modification of L-NMMA, *viz*, synthesis of its *N*-oxide (**4**), has given a fairly efficient iNOS inhibitor (with a selectivity 10 times higher than that for eNOS and 6 times higher than that for nNOS). A specific feature of compound **4** is its ability to inhibit nitric oxide production upon administration of lipopolysaccharides (endotoxins);[44a] this attests, apparently, to a high selectivity of the *in vivo* inhibition by L-NMMA *N*-oxide.



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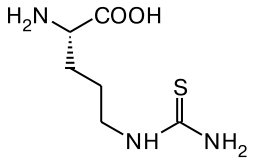
As regards L-NMMA itself, which was noted above to be a nonselective inhibitor of the constitutive and inducible NOS, mention should be made of a study[45] reporting both the synthesis and detailed analysis of the biological action of this compound. A close L-NMMA analog, *N*-propyl-L-arginine, inhibits the neuronal isoform (nNOS), moreover, with a pronounced selectivity.[44b] This compound is presumably a potential drug for treatment of Alzheimer´s disease and other neurodegenerative diseases.

3. Other amino acid-type NOS inhibitors

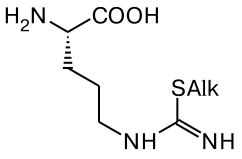
Arginine and citrulline analogs with functional substituents able to bind to heme are efficient inhibitors of NO synthases.[46] This group includes thiocitrulline and its analogs. Study of the inhibition kinetics showed that thiocitrulline is a competitive inhibitor of arginine oxidation (K_i = 0.06 μmol L^{-1} for nNOS and 0.36 μmol L^{-1} for iNOS).[5] In addition, these compounds prevent the for-

* Linear peptides (α, 13 amino acids, a central antipyretic possessing a cardiovascular effect and acting on the central nervous system; β, 18—22 amino acids; γ, 12 amino acids, endorphin antagonist[14,15]).

mation of active forms of oxygen. *S*-Alkyl derivatives of L-thiocitrulline (**5a**) exhibit *in vitro* a higher selectivity with respect to nNOS than **5a** itself. However, this selectivity cannot be observed *in vivo*, because, for example, *S*-ethylthiocitrulline exhibits in the corresponding experiments a pronounced pressor effect (blood pressure increase), which is not apparently related to nitric oxide.[3]



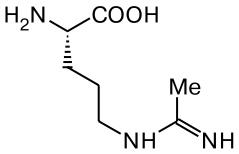
5a



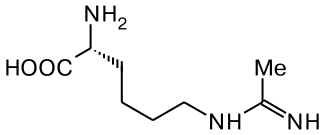
5b,c

Alk = Me (**b**), Et (**c**)

S-Methyl- (**5b**) and *S*-ethylthiocitrullines (**5c**) are active and reversible inhibitors for any NOS isoform; both are much more active with respect to the neuronal than to the endothelial or inducible form[47] (the effects of **5b** and **5c** toward nNOS are 10 and 50 times, respectively, more pronounced than on eNOS). Binding of these compounds to the enzyme competes with binding of arginine and proceeds rather slowly. The relatively low formation rate of the substrate—enzyme complex is attributable[48] to slow conformational changes in the enzyme structure needed for effective binding or to the fact that the minor conformer of the inhibitor is involved in the process. *S*-Methyl- and *S*-ethylthiocitrullines inhibit by 97% the NADPH-dependent oxidase activity of the neuronal isoform. This property is by no means inherent in all inhibitors. For example, L-NNA and L-NAME posses this feature, while L-NMMA or L-iminoethylornithine do not inhibit NOS-catalyzed oxidation.



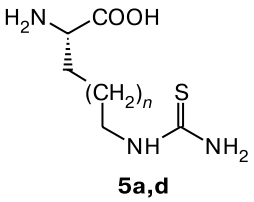
N-Iminoethyl-L-ornitine



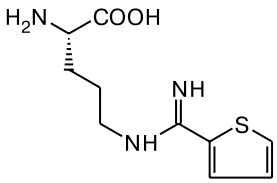
N-Iminoethyl-L-lysine

Note that *N*-iminoethyl-L-lysine is also a selective inhibitor of the inducible NOS form.[49]

A study[43b] of thiocitrulline (**5a**) and homothiocitrulline (**5d**) derivatives and other similar compounds incorporating a sulfur-containing fragment (for example, a thiophene ring) showed that they mainly inhibit the neuronal isoform and, correspondingly, they have been recommended as potential drugs against hypotension.

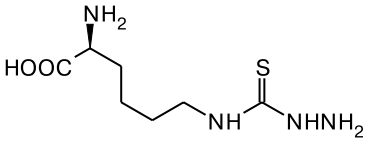


5a,d



n = 1 (**a**), 2 (**d**)

Substituted homothiocitrulline derivatives (**6—8**) were found to inhibit mainly the inducible form of NOS. However, they also affect the constitutive forms of the enzyme and therefore, their selectivities are not very high.[50a]



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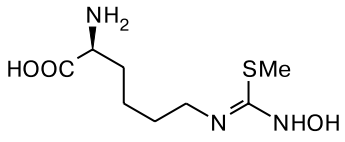

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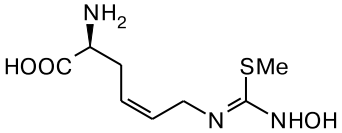


8

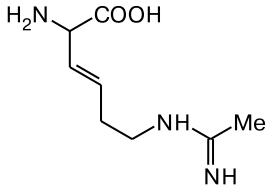
A study of the effect of selective and nonselective inhibitors of nitric oxide synthesis on a systemic inflammatory process and damage of organs and tissues caused by introduction of lipopolysaccharides (endotoxins) showed[51] that nonselective inhibitors enhance both necrosis and apoptosis* (hepatic), while selective ones enhance only the apoptosis, which is a weighty reason supporting the search for selective inhibitors as compounds whose use is favorable as regards toxicity.

Examples of nonselective amino acid inhibitors of NO synthase are *N*-iminoethyl-3,4-dehydro-L-lysine (**9**), which is being considered[52a] as a remedy suppressing the immune responses upon organ transplantation, and a series of C-substituted arginine and iminoethylornitine derivatives (**10**, **11**), recommended in the case of septic shock.[52b]

Other lysine and thialysine (**12**, **13**) derivatives also proved to be selective with respect to iNOS. These inhibitors can be used against septic or toxic shock as immunosuppression drugs (suppression of foreign tissue rejection) in the transplant therapy.[42b]

The selectivity of action of amino acid inhibitors (**14**) used against systemic hypotension associated with septic

* Necrosis is the irreversible termination of the vital activity of particular tissues. Apoptosis is an active cell destruction process. The purpose of this cell death is to remove undesirable cells that have lost their functions during its development or due to aging and the cells potentially hazardous for the organism (mutant cells, virus-infected cells and so on).



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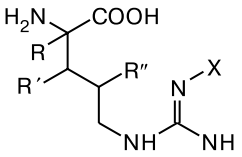

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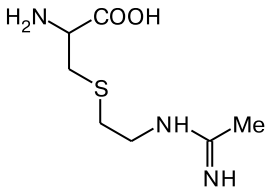
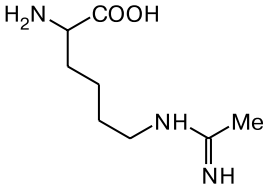


11

R = R′ = R″ = H, Me; X = Me, NH_2, NO_2



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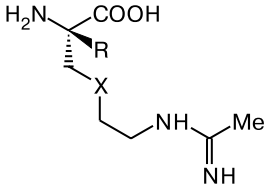
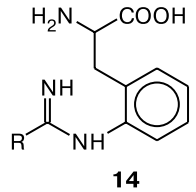


13

R = Me, X = CH_2; R = CH_2OH, X = CH_2; R = H, X = S

shock is dependent on NO synthase localization. It is significant that these compounds contain phenylalanine residues rather than aliphatic amino acids, *i.e.,* the search for NOS inhibitors allows rather substantial structural changes.[43c]



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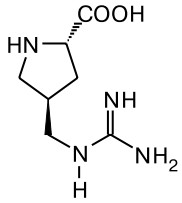


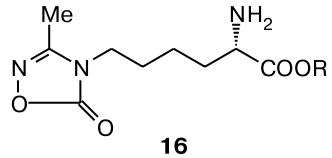
15

R = NH_2, SMe, NHMe, 

The proline analogs of arginine (**15**) acting as iNOS inhibitors are recommended for treating immunoinflammatory diseases such as rheumatoid arthritis. A necessary condition for the activity is the use of L-proline, while the configuration of the other chiral center is not very significant for manifesting the activity.[42c]

Oxadiazoles with containing amino-acid substituents (**16**, **17**) also display a selective activity toward inducible NO synthase.[50b]

Sulfur-containing amino-acid amidines **18**, **19**, which are selective iNOS inhibitors, have been proposed[53a] for



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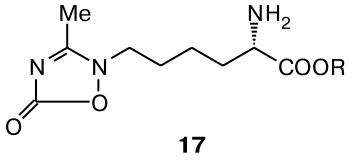
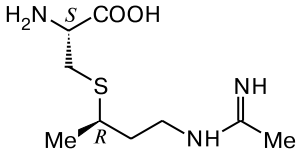
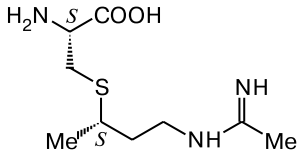


17

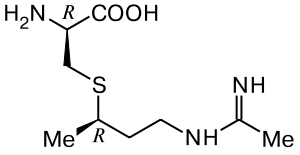
the therapy and prevention of arthrites, migraines, and pathologies associated with ileus (bowel obstruction) (the selectivity is 100 times as high as that toward eNOS; the compounds are inactive with respect to nNOS).



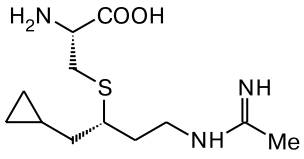
(*R,S*)-**18**



(*S,S*)-**18**

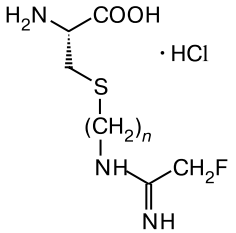


(*R,R*)-**18**

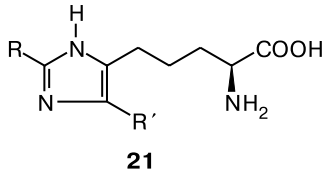


19

An important feature of this type of compound having fluorine-containing substituents (**20**)[50c] is that they are selective iNOS inhibitors characterized by an extremely long time of action *in vivo*.



20, *n* = 2, 3

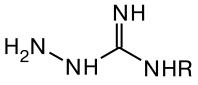


21

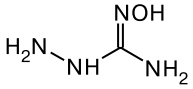
Imidazolyl amino acids (**21**) also inhibit inducible and neuronal nitric oxide synthases.[54]

4. Nonamino acid-type NOS inhibitors: guanidines and amidines

Many guanidine derivatives are well-known to be efficient nitric oxide donors.[2] However, a number of guanidine derivatives exhibit a clear-cut antagonism relative to NO synthases. *N*-Aminoguanidine (**22a**) and its derivatives stand out in this respect.



22a,b

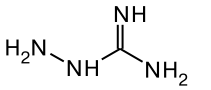


23

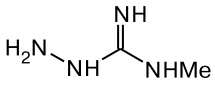
R = H (**a**), Me (**b**)

In most *in vitro* systems, aminoguanidine and L-NMMA are equally effective for inhibiting the inducible isoform but the former is an order of magnitude less active relative to constitutive forms. In animal models, aminoguanidine decreases the disease severity at inflammations and septic shock and increases the survival rate upon administration of endotoxins. The activity profile of aminoguanidine is favorable for a patient, but the necessity of using high doses for attaining the effect is an obvious drawback. With these doses, inhibition of constitutive NOS as well as catalase and other copper- and iron-containing enzymes also becomes noticeable, which results in various toxic effects related, in particular, to accumulation of active forms of oxygen.[3,55] It has been shown[56] that due to selective inhibition of iNOS, aminoguanidine prevents interleukin-1β-induced hyperglycemia and a decrease in the insulin production by the pancreas. The most important aspect of these effects is that it is the inducible enzyme that is selectively blocked; L-NNA, which is selective for different, constitutive isoforms, exerts this effect only to a minor extent. The *N*-hydroxy derivative of aminoguanidine (**23**)[57] functions as a strong iNOS inhibitor and can be regarded as a potential drug against dysfunctions caused by endotoxins.

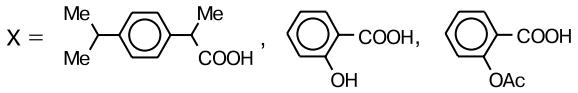
The salts formed by aminoguanidine and *N*-methylaminoguanidine (**22a**·X, **22b**·X) with antiinflammatory drugs having acidic groups, for example, with ibuprofen and salicylic or acetylsalicylic acid,[58a] were found to be dual inhibitors, *i.e.*, inhibitors of both nitric oxide synthases (mainly iNOS) and cyclooxygenases (COX-1 and COX-2).



22a



22b



A selective inhibitor of the cytokine-induced transport of arginine and nitric oxide production by the inducible isoform has been found in the series of hydrazones of the type **24**, containing aminoguanidine fragments. Moreover, this compound exhibits no effect toward eNOS. It also displays a high level of antiinflammatory activity and a protective effect from endotoxins and, in addition, acts as an inhibitor of the cytokine-induced formation of nitric oxide in activated macrophages.[43d]

Potential drugs against septic shock, hypotension, brain ischemic diseases, rheumatoid arthrites, ulcerative colitis, and insulin-dependent diabetes have been found among iNOS inhibitors of the series of substituted arylaminoguanidines and arylguanidines (**25—27**).[42d]

Of other guanidine derivatives, we would like to mention *N*-tetramethylene-*N*´-arylguanidines **28**, **29**.[43e]

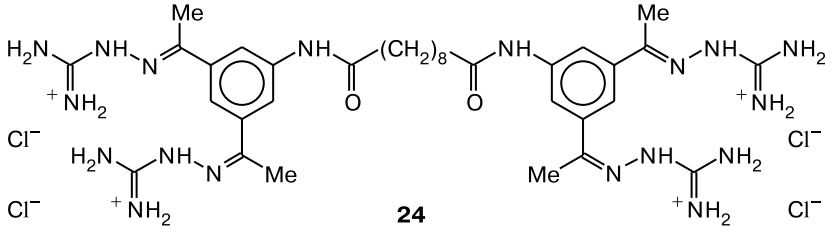
As selective nNOS inhibitors, these compounds are meant for the therapy of neurodegenerative diseases, improvement of the stomach contractive ability, and against various inflammatory pathologies.

Nonamino acid nitroguanidines *S*- and *R*-**30** inhibit n- and i-NOS (predominantly, nNOS) and can be used for the therapy of vascular system diseases, coronary spasm, cerebral blood flow disturbance, atherosclerosis, diabetes, and sepsis.[43f]

Some nitroguanidines, derivatives of amino acid esters (**31**), are used for the same purposes.[58b]

Amidines **32—36** are selective inhibitors of the neuronal NO synthase, whose major application is for treating various neurodegenerative diseases.[43g]

A large number of amidines (**37—52**) with highly diversified structures functioning as selective iNOS inhibitors are documented.[42e,43h,i,44c—i,50d,e,53b,58c—f]



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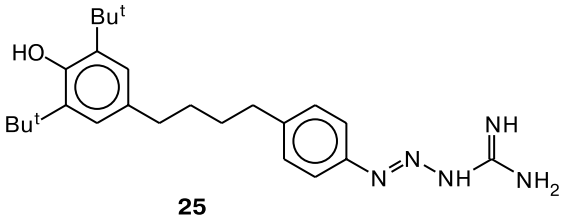

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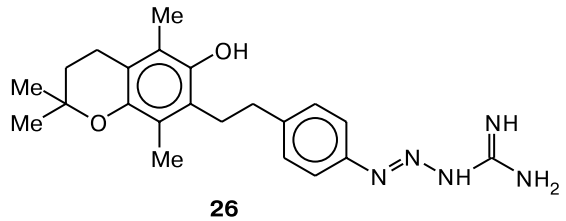

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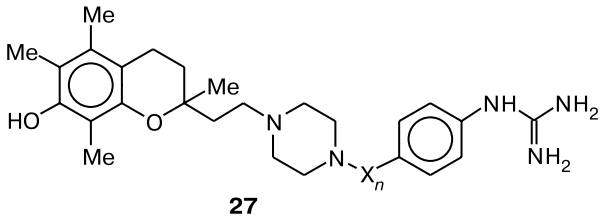

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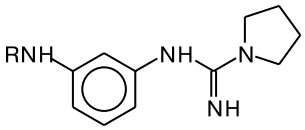
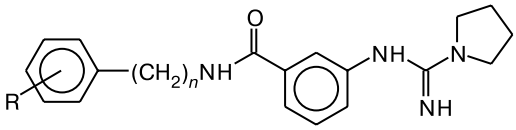
n = 2, X = CH_2; n = 1, X = CH_2O; n = 0



28

R = COMe, $COCHMe_2$, p-Cl-C_6H_4, o-Cl-C_6H_4



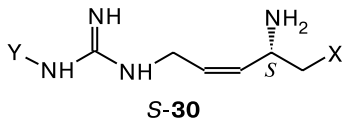
29

R = H, n = 1—3; R = p-Me, p-F, o-Cl, n = 2

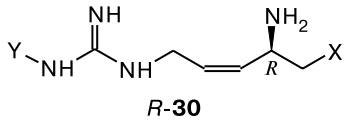
However, other amidines that inhibit efficiently NO synthases either do not show selectivity with respect to iNOS or even inhibit selectively another isoform, namely, nNOS. Thus cyclic amidines **53** have only limited selectivity with respect to iNOS but their activity toward nNOS is only 2.5 times lower than that toward iNOS (these compounds are 250 times less active with respect to eNOS).[58g]

Amidines of the 3,4-dihydroisoquinoline series and related systems (**54**, **55**) also inhibit the inducible and neuronal enzymes.[44j]

A whole series of amidines of various types function as selective nNOS inhibitors (**56—60**).[50f—j] However, no data concerning the structure—activity relationship in these series of compounds have been reported and no clear criteria have been elaborated to determine what compounds would be tropic to particular NO synthase isoforms.

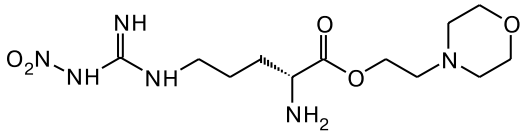


S-**30**



R-**30**

Y = NO_2, NH_2, NHMe; X = OH, OMe



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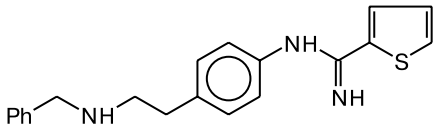

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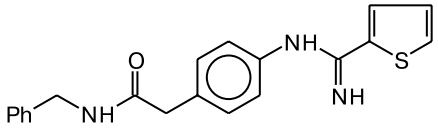

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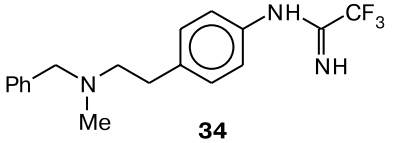

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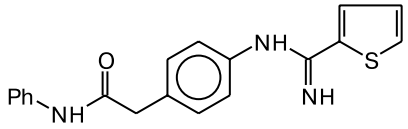

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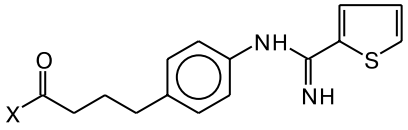

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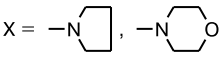
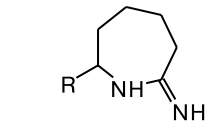
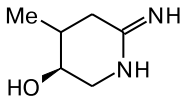
X = 



37

R = *cyclo*-C_6H_{11}, Bu, C_5H_{11}
H_2C=CH—$(CH_2)_2$—



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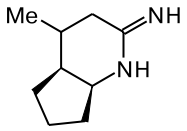

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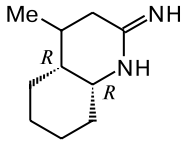
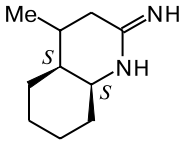
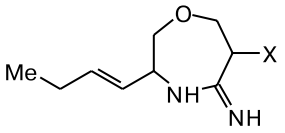


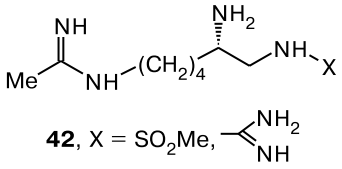
(*R,R*)-**40**



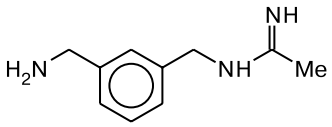
(*S,S*)-**40**



41, X = H, OH



42, X = SO_2Me,



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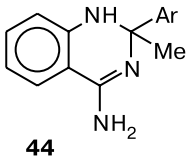

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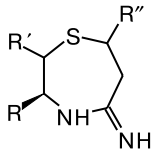
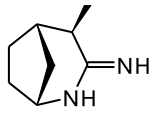
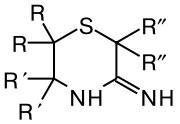


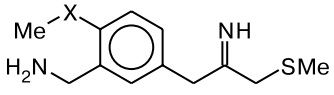
45, R, R′, R″ = H, Alk



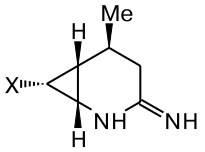
46



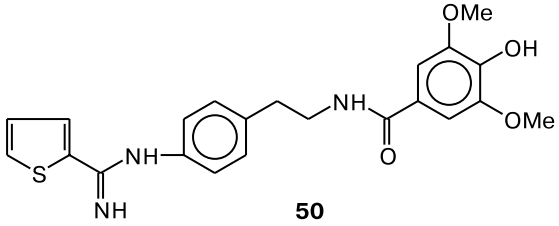
47, R, R′, R″ = H or Alk



48, X = CH_2, O



49, X = H, Cl



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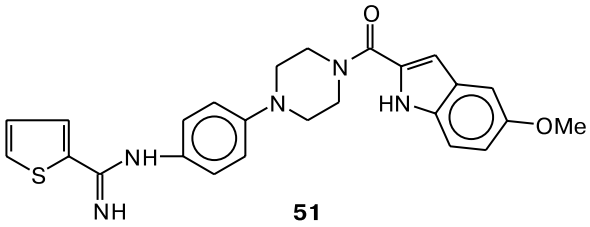

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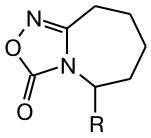
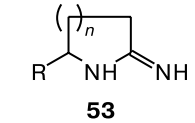


52, R = H, $CH_2CH_2NH_2$

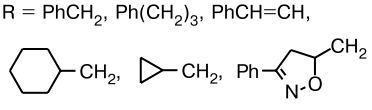
A separate group of amidines is represented by S-substituted isothioureas, which are often found to be highly efficient NOS inhibitors. Like L-NMMA, these compounds damage the systems surrounding the heme and thus violate its interaction with the substrate. It is rather important that relatively slight variations may result in a substantial level of their selectivity with respect to different isoforms.[3] Indeed, *S*-isopropylisothiourea exhibits clear-cut selectivity for iNOS *in vitro*, while *S*-methylisothiourea shows a pronounced selectivity for iNOS relative to nNOS *in vivo*, although this difference is leveled out *in vitro*. Bis-isothioureas are efficient and selective iNOS inhibitors; however, the use of these compounds as drugs is hampered by their poor ability to penetrate bio-

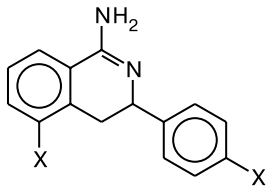


53

n = 1—3

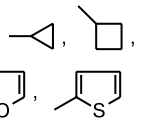
R = $PhCH_2$, $Ph(CH_2)_3$, PhCH=CH,

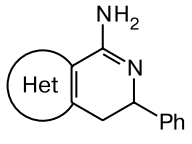




54

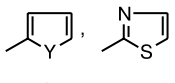
—X = —H, —F,



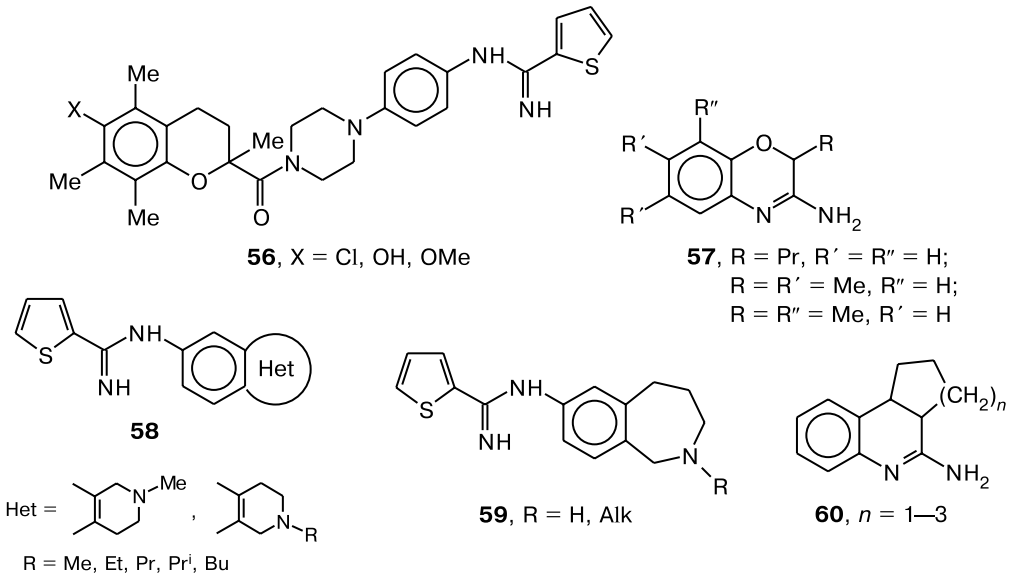


55

Het =

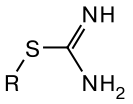


Y = NR, S



logical membranes and high toxicity. A comparison of the activities of amidines and guanidines provides the following sequence of inhibitory action with respect to iNOS:[59] *S*-ethylisothiourea > *S*-methylisothiourea > 2-iminopiperidine > butyramidine ≃ *N*-methyl-L-arginine > > ethylguanidine > methylguanidine > *O*-methylisourea. In the series of "conventional" amidines, the highest activity toward iNOS is found for 2-iminopiperidine.

S-Ethyl- (**61a**) and *S*-isopropylisothioureas (**61b**) exhibit high activity in blocking both the endothelial and neuronal isoforms without noticeable selectivity, whereas *S*-methyl- (**61c**) and *S*-β-aminoethylisothiourea (**61d**) predominantly inhibit iNOS.[60,61]



61a—d

R = Et (**a**), Pr^i (**b**), Me (**c**), $H_2N(CH_2)_2$ (**d**)

According to a publication,[60] these iNOS inhibitors could serve as a convenient tool for investigating the role of NO in various pathologies and could be used to treat the diseases* caused by overproduction of NO by this isoform. *S*-Methylisothiourea is the most active iNOS inhibitor, which protects animals from blood circulation disturbances and organ dysfunctions caused by endotoxins and increases the survival rate upon the septic shock.[61] The iNOS-selective inhibitor do not entail side effects as severe as eNOS inhibitors (sharp vasoconstriction, organ ischemia, and enhanced inhibition of platelet** and neutrophil*** adhesion). In a special study[62] devoted to comparison of *S*-ethyl- (SEITU) and *S*-β-aminoethylisothioureas (SAEITU), the latter was shown not only to inhibit iNOS but also to prevent expression of this isoform in macrophages. Both compounds are equally active as inhibitors but SAEITU not only blocks iNOS but also inhibits the iNOSmRNA translation* to iNOS and accelerates the post-translational degradation of the apoenzyme (protein component) of the inducible isoform, *i.e.*, it exhibits more versatile activity. This provided the conclusion that SAEITU should not be used if the researcher´s goal is only selective inhibition of the catalytic activity of iNOS. In the same study, L-NMMA, L-NNA, L-NAME, and aminoguanidine were compared with *S*-substituted isithioureas; the latter group was shown to be 30—100 times more potent inhibitors. Indeed, in inhibiting the nitric oxide production by iNOS of lipopolysaccharide-activated macrophages, the effective inhibitor concentrations EC_{50} are 7±1 and 9±2 μmol L^{-1} for *S*-ethyl- and *S*-β-aminoethylthioureas and 580±42, 760±68, and 240±28 μmol L^{-1} for *N*-methylarginine, *N*-nitroarginine, and aminoguanidine, respectively.

A number of selective iNOS inhibitors (**62—68**) have been described to a larger or smaller extent.[42f,g,52c,58h]

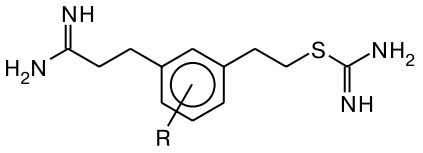
Discussion of the problem of interaction of *S*-alkylisothiourea with the enzyme active site has shown[43j] that these compounds are markedly more active than arginine derivatives. For example, *S*-methylisothiourea is a 500 times more active iNOS inhibitor than *N*-methyl-L-arginine, attesting to the key role of the S atom in binding to the enzyme, which depends apparently on the affinity to the heme. The ability of NOS inhibitors to increase blood pressure can be employed against hypotension in the case of septic shock (see Ref. 33). The selectiv-

* Inflammations, circular shock caused by blood flow disturbance, or cancer.

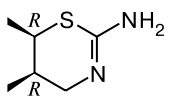
** Platelet is a regular blood element participating in the blood clotting process.

*** Neutrophil is a kind of leucocytes.

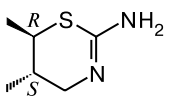
* Translation is the process of using the genetic information from mRNA for protein synthesis (formation of an appropriate amino acid sequence).



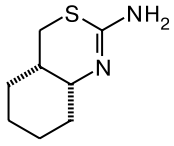
62



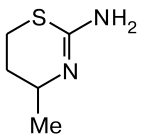
(*R,R*)-**63**



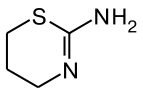
(*R,S*)-**63**



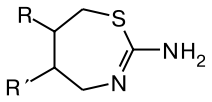
64



65



66



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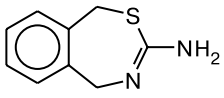
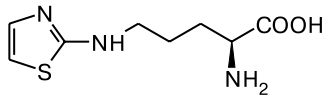


68

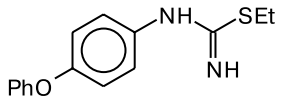
R = H, Ph, R´ = H; R = H, R´ = Ph

ity of the inhibitor action determines the possibility of realization of the effects needed for the therapy of particular diseases. For instance, in acting upon nNOS for preventing neurodegenerative processes, strokes, *etc.*, the absence of a pronounced effect on eNOS accompanied by a sharp decrease in blood pressure is an important factor. The same is true for iNOS. It is not clear yet in what cases, when acting on the inducible isoform, one should avoid inhibiting the neuronal NOS. Therefore, compounds with a low selectivity with respect to NO synthase isoforms,[43j,63] for example, thiazole derivative **69**, are often considered to be potentially useful.

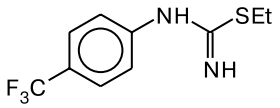


69

A hypothetical model of functioning of nNOS implies activation of the isoform in the case of the cerebral ischemia; the excessive production of nitric oxide, *i.e.*, a compound cytotoxic with respect to the surrounding neurons, increases under these conditions.[64,65] Thus, nNOS inhibitors should protect organs from ischemic damages. However, the use of *N*-nitro-L-arginine in various models of ischemic disease brings about a series of side effects caused, apparently, by the fact that this compound is equally active toward eNOS.[66] This results in a number of undesirable consequences such as vasoconstriction, decreased blood flow, and reduced oxygen saturation of ischemic tissues, which are related to eNOS inhibition. This accounts for the necessity of looking for selective inhibitors of the neuronal enzyme. *S*-Alkylisothioureas such as **70—72** prepared by *S*-alkylation of thiocitrulline are examples of compounds proposed[48] as such inhibitors.



70



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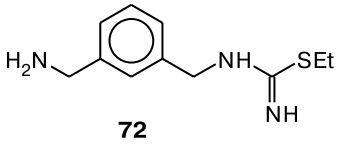
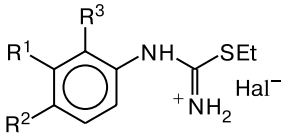


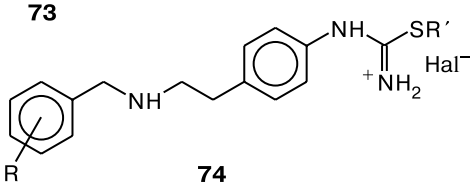
72

They tend to exhibit a ~20 times higher activity toward the neuronal than toward the inducible isoform.[43g]

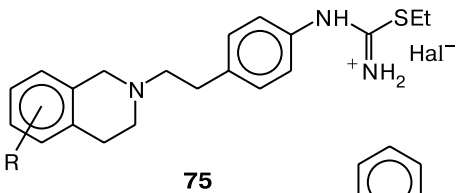
Selective nNOS inhibitors were found among *N*-arylisothiouronium salts **73—77** with aminoalkyl substituents in the aromatic ring.[42h—j,53c]



73



74



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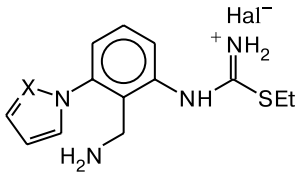
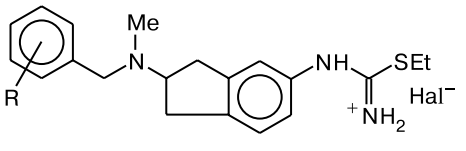


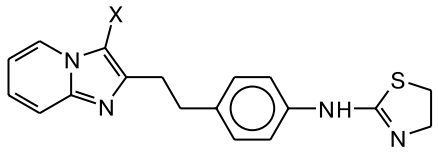
76, X = CH, N



77

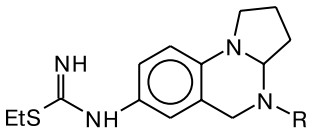
R^1 = $H_2NCHAlk$ (Alk = Me, Et, Pr^i, Bu^t), $H_2NCH_2CH_2$;
R^2 = H, NMe_2, OEt; R^2 = H, Me;
Hal = Cl, Br, I

The presence of basic centers in the substituents appears to be obligatory, but these should not necessarily be aminoalkyl groups; they can also be incorporated in heterocycles, for example, as in compounds **78**, which are selective nNOS inhibitors.[58i]



78, X = H, Cl

Unfortunately, such a simple criterion for nNOS selectivity as the presence of a basic fragment does not obviously exhaust the problem; indeed, tricyclic compounds **79** and **80**, containing an isothiourea fragment and amine or amidine residues incorporated in a cyclic system, inhibit with equal efficiency the neuronal and inducible isoforms, although they act much more strongly (by a factor of 75) on eNOS.[44k]



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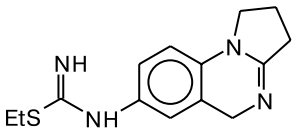
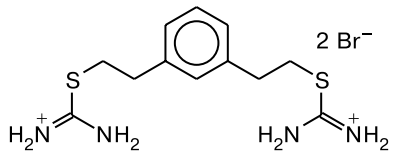


80

The presence of the bis-amidine system does not ensure the affinity to exactly the neuronal isoform either. Moreover, bis(isothiouronium) salt **81** displays a rather high level of selectivity for iNOS (the inhibition is 190 times as high as for eNOS). Note that a similar activity profile is characteristic of a bis-amidine, 1,3-bis-acetamidinomethylbenzene (**82**). Of the inducible enzyme (iNOS) inhibitors found to date, *N*-(*m*-aminomethylbenzyl)acetamidine (**83**) (designated in the literature as 1400W) is the most selective.[67]

Here it is pertinent to mention a hypothesis forwarded long ago[68] according to which the biological effects of the isothiouronium salts, guanidinium salts, and amidines (which, naturally, had nothing to do at that time with nitric oxide, whose role was discovered a quarter of a century later) were due to positive charge delocalization mimicking the solvation of potassium and sodium ions and to binding to receptors of these ions. This idea is supported by the fact that isothioureas inhibit not only NOS but also Na^+-binding to the renal K,Na-ATPase. Presumably,[67] when 1400W binds competitively with arginine to NOS, the acetamidine fragment interacts with the "arginine site" of the enzyme, while the amine fragment interacts with a site other than the enzyme active site rather than with the "amino-acid site". The kinetics of binding of 1400W to NOS, as often observed for many inhibitors of this enzyme, implies a two-step process, the selectivity for iNOS being determined by the second, slow step. Since this step is NADPH-dependent, the selectivity for iNOS is determined by the fact that the formation of an enzyme—substrate complex can change the electron transport from NADPH to the heme iron, accelerate its reduction, and induce inactivation of the inducible isoform. It remains obscure why these processes do not affect constitutive isoforms.



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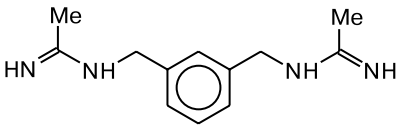

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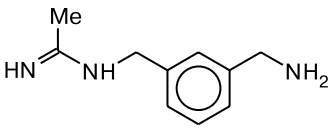
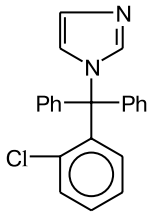


83

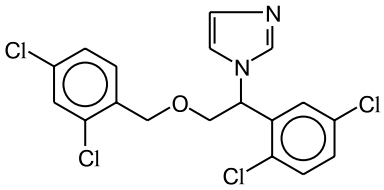
5. Derivatives of heterocycles as NOS inhibitors

It has been already discussed that compounds capable of binding to the heme inhibit the enzyme activity. In other words, heme ligands such as carbon monoxide, cyanide anion, and nitric oxide itself, as well as some heterocyclic compounds including imidazole, 2-phenylimidazole, and antimycotic drugs myconazole, ketoconazole, and clotrimazole inhibit all of the NOS isoforms.[69—71]

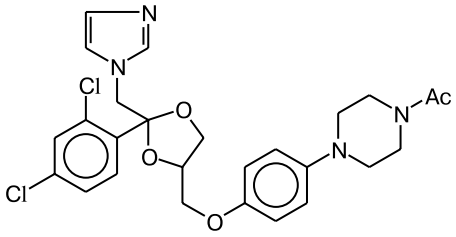
These compounds bind to the heme through interaction with the imidazole fragment, and the antimycotic drugs act additionally as antagonists to calmodulin, the protein regulating the nNOS activity. In a number of cases, a substantial isomorphous selectivity is attained. For instance, the inhibition constants (K_i) for nNOS, eNOS, and iNOS by 2-phenylimidazole are 38, 50, and 0.7 μmol L^{-1}, respectively.[25] Imidazole is known to be a cytochrome P450 antagonist.[69] Since, as noted above, NOS largely resemble cytochrome P450 in structure, intensive search for NO synthase inhibitors has been per-



Clotrimazole

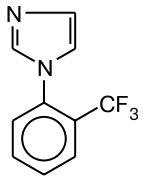


Myconazole

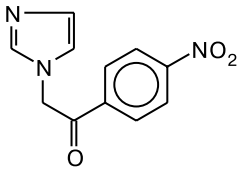


Ketoconazole

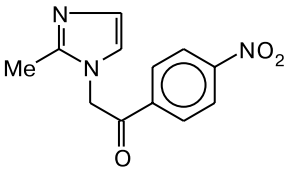
formed among derivatives of this heterocycle, and 1-(2-trifluoromethylphenyl)imidazole (TRIM) proved to be an active nNOS inhibitor (it is also active, although to a lesser extent, with respect to eNOS).[72] *N*-*p*-Nitrophenacylimidazoles **84a,b** proved to be selective inhibitors of the neuronal isoforms; some aspects of the mechanism of their action were the subject of a publication.[73]



TRIM



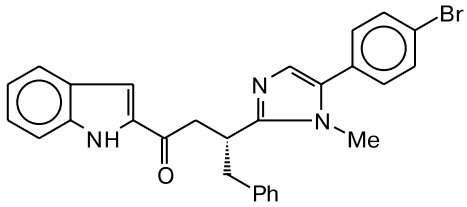
84a



84b

These phenacyl derivatives inhibit nNOS without competing with arginine but competitively with tetrahydrobiopterin (BH_4). One approach to the synthesis of selective inhibitors of the neuronal isoform is the search for compounds that react selectively with BH_4. The inhibition constants of NOS isoforms were found for compounds **84a,b**, namely, the K_i values for nNOS are 105 and 74 μmol L^{-1}, rerspectively (note for comparison that K_i of nitroarginine at the same concentration is 0.5 μmol L^{-1}), while for the inducible NO-synthase, K_i is 1.100 and 1.050 μmol L^{-1}, respectively.

Complex imidazole-containing compounds **85** and **86** are NOS inhibitors,[44l,53b] but no detailed data concerning their selectivity have been reported.



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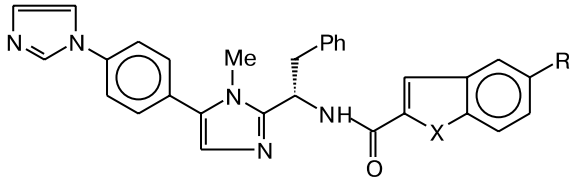
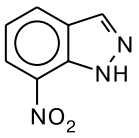


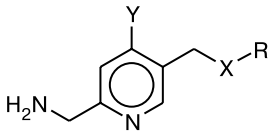
86, R = H, NO_2

7-Nitroindazole is a relatively selective nNOS inhibitor.[25,53d]



This compound binds to nNOS, eNOS, and iNOS with inhibition constants K_i = 0.16, 0.8, and 1.6 μmol L^{-1}, respectively.

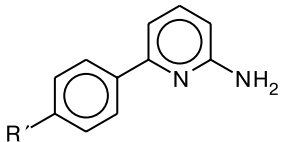
An antagonistic action was found for 2-aminopyridines **87**, **88**.[44m—o,50k,l,53d—f]



87

R = Me, Et; Y = H, Me

X = O, S, CH_2



88

R´ = H_2N

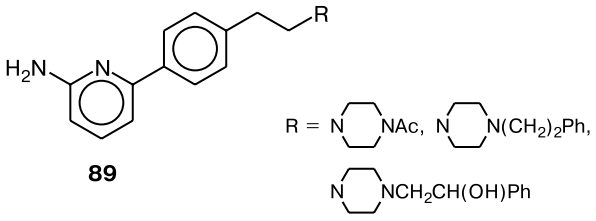
These compounds inhibit iNOS and nNOS.

Both selective inhibitors of the neuronal isoform (**89—91**)[44o,53d,e] and the inhibitors exhibiting approximately equal levels of inhibitory activity with respect to iNOS and nNOS (**92**) have been found in the aminopyridine series.[50k,l]

2-Aminopyridine derivatives (**93**)[53f] not only block nNOS but also inhibit lipid peroxidation by decreasing the concentration of the active oxygen forms.[74]

Selective iNOS inhibitors can be found among various heterocyclic compounds; these are, *e.g.*, 5-chloro-3-morpholinomethylbenzooxazol-2-one (**94**)[58j] and tetrahydroindole derivatives (**95a,b**), which, in addition, inhibit cyclooxygenase 2.[50m]

Representatives of pyridothienotriazine derivatives (**96**, **97**) decrease the levels of NO and prostaglandins by



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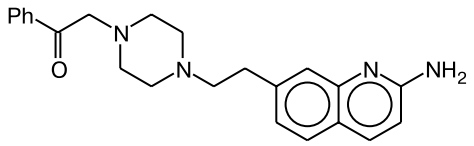

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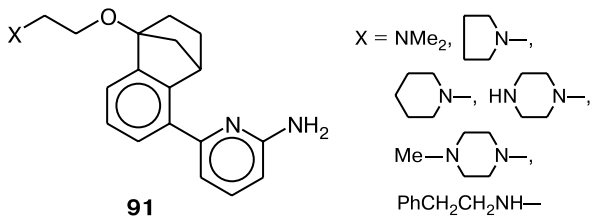

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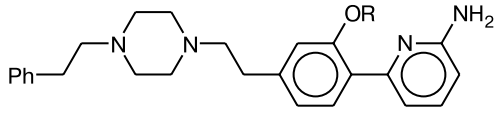

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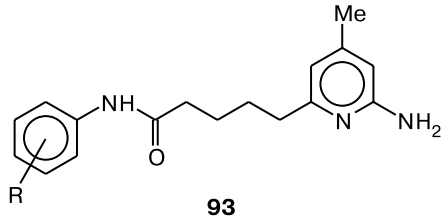
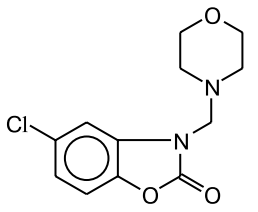


93

R = OH, OMe, NMe_2, Bu^t, Pr^i



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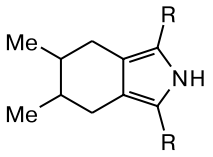
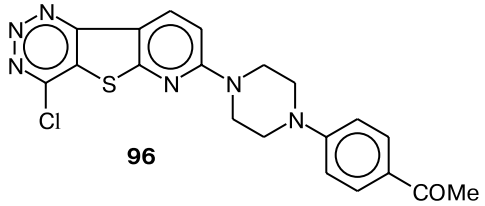


95a,b

R = Ph (**a**), *p*-FC_6H_4 (**b**)



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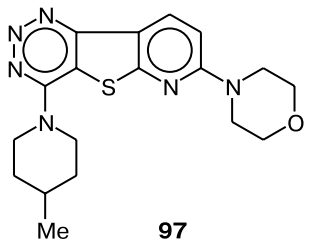
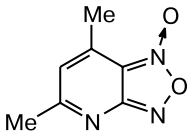


97

decreasing the iNOS and cyclooxygenase COX-1 and COX-2 expression.[75]

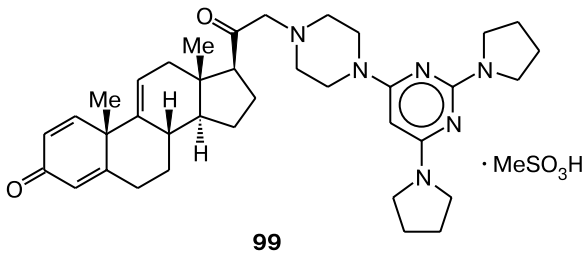
Pyridofuroxans (**98**) proved to be active and rather potent inhibitors of the inducible isoform (which is inhibited 25 times as strong as nNOS).[44p]

This is an unusual situation because many furoxan derivatives are well-known[2] to be efficient NO sources; the fact that compounds with similar structures can exhibit both donor and inhibitory properties deserves, undoubtedly, special attention.



98

Potent NO synthase inhibitors have also been found among steroidal compounds containing heterocyclic fragments as substituents. As an example, we can cite tirilazad mesylate (**99**), which is currently under clinical investigations as an anticataract drug and a remedy against various neuronal disorders. The mechanism of its action is attributed to both NOS inhibition and the inhibition of the lipids peroxidation.[76]



99

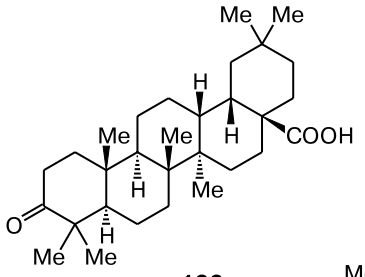
6. Miscellaneous inhibitors

Triterpenoids **100—102** inhibiting both iNOS and cyclooxygenase 2 (COX-2) have been reported.[77]

All nitric oxide synthases require that NADPH-dependent FAD and FMN be involved in their catalytic action. Correspondingly, inhibitors of these coenzymes should also act as NOS inhibitors. Indeed, compounds that display antagonism to the action of NADPH and FAD also block NOS. Iodonium salts **103—105** are examples of such compounds.[78]

The inhibitory activity of ebselen (**106**) is prevented by administration of thiols.

It was concluded that ebselen can modify the most important thiols incorporated in constitutive NOS to give



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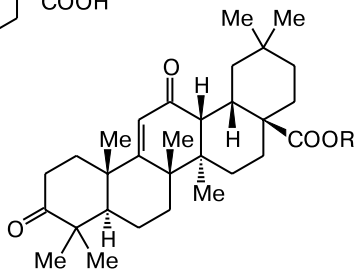
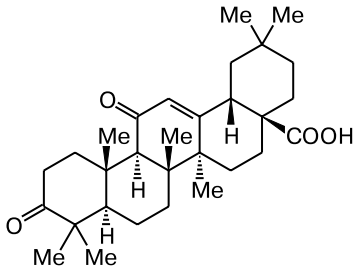


101a,b

R = H (**a**), Me (**b**)



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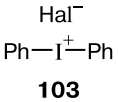

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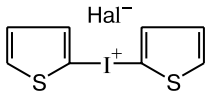

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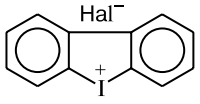

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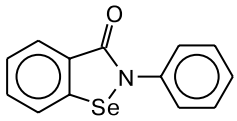
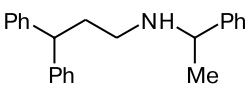


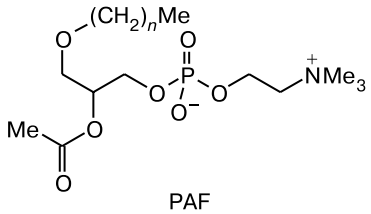
106

selenosulfides and that exogenous thiols can protect enzymes by intercepting and trapping ebselen. Ebselen exhibits the highest activity with respect to eNOS because it mainly reacts with thiols from endothelial cells, although in some other cells, it is also an efficient iNOS inhibitor.[5] As noted above, constitutive NOS are activated and regulated by calcium ions through reversible binding to calmodulin. Therefore, inhibitors of the calmodulin functions should have a pronounced influence on the activity of constitutive NOS. A study of these inhibitors, for example, fendilin (**107**), showed that they really inhibit eNOS and nNOS but do not affect iNOS.

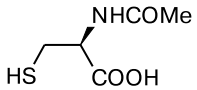


107

According to a hypothesis, during an initial period of a shock,* nitric oxide produced by constitutive synthases plays a favorable role in the therapy.[79] However, in a subsequent phase, hyperproduction of nitric oxide by the inducible isoform is responsible for toxic phenomena. Thus, it follows that selective inhibition of iNOS but not the constitutive forms is favorable for treatment of an experimental shock.[80,81] The iNOS induction by bacterial endotoxins is due to the release of many proinflammatory cytokines such as interleukin-1(IL-1), tumor necrosis factor (TNF-α), interferon-γ (IFN-γ) as well as the platelet-activating factor (PAF).



PAF



NAC

n = 15—17

These mediators are linked to the corresponding surface cell receptors; they activate tyrosine kinase and then transcription of the nuclear factor kB (NFkB);** this results in transcription and translation of iNOS proteins in various organs leading to excessive production of nitric oxide from arginine. *N*-Acetylcysteine (NAC) is an example of selective inhibitor of the inducible isoform.

The administration of this compound into the organism induces the iNOS inhibition and decreases the content of dinitrosyl(iron) dithiolate complexes; thus, NAC protects the liver from damage by the enzymes that cause NO over-production. It was shown that NAC exhibits a strong inhibitory action on the expression of the iNOS gene.[82]

7. The therapeutic potential of NOS inhibitors

The activities of nitric oxide and, hence, of inhibitors of its formation are so versatile that merely the inhibition of NOS isoforms can entail serious problems. In this connection, preparation of selective inhibitors acquires ever increasing importance. The stability of hemodynamics is so crucial for the organism that the inhibitors used for the therapy of various diseases should not be antagonistic to eNOS.[40] Although it is obvious that over-production of nitric oxide results in a number of metabolic and hemo-

* The organism response to the action of extraordinary stimuli characterized by development of severe disorders of blood circulation, respiration, and metabolism.

** An inducible DNA-binding protein involved in inducing the transcription of many genes in macrophages and proteins by the inducible NOS.

dynamic disturbances, the excessive eNOS inhibition, for example, in the case of a septic shock can provoke the release of endogeneous vasoconstrictors (pressor factors) and organ lesion in the absence of NO. It should be borne in mind that after its synthesis in postsynaptic endings, NO is free to diffuse back to presynaptic endings; thus, it can be defined as a retrograde mediator, required, for example, for arrangement of the memory.[5] The inhibition of formation of the compound that acts as a neuromediator in neurons belonging to neither adrenergic nor cholinergic systems should be done with caution. Yet another problem[5] caused by the versatility and ambiguity of NO functions is the regulation of its concentration in the organism by changing the Ca^{2+} or calmodulin content. This may entail undesirable consequences, because the use of this complex is not limited to the catalytic function of NOS alone.

Now we discuss some pathologies directly related to excessive production of nitric oxide (see Refs 3, 5).

As noted above, NOS inhibitors are favorable against the septic shock caused by administration of endotoxins. Therefore, the use of NOS antagonists is currently considered to be a correct approach to treatment of septic shock.[3]

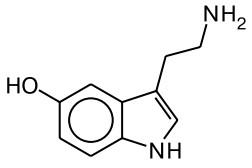
Vessel thrombosis and (or) cerebral hemorrhage lead to a loss of blood flow, hypoxia, metabolic changes, glutamate ejection, and an increase in nitric oxide release, which is due to nNOS activation in the early stages and to expression by iNOS in the later stages. The role of nitric oxide in the focal ischemia is contradictory: it can be both a protective and a damaging agent. It has been reported[3] that combined use of inhibitors of neuronal and inducible isoforms can be quite useful for treating cerebral ischemic diseases. However, NO formed upon nNOS catalysis can cause neurotoxicity and enhance damage of neurons. The shock caused by occlusion of internal arteries is known[83] to be due to excessive NO production by the inducible isoform and by its deficient production by the endothelial isoform. The use of *S*-ethylisothiourea (iNOS inhibitor) with an NO donor of the sydnonimine series markedly ameliorates the state caused by the shock.

The excessive formation of glutamate and nitric oxide causes injuries similar to those observed in the case of Parkinson´s, Alzheimer´s, and Hangtinton´s diseases (the last one is a hereditary nervous system disease). Experiments on animals have shown that neurodegenerative diseases are stopped by NOS inhibitors. Thus the use of 7-nitroindazole improves motile functions and cognitive (pertaining to memory) functions and removes brain injuries resulting in Hangtinton´s disease. Alzheimer´s disease appears to be also provoked by the fact that β-amyloid plaques characterizing this disease induce the iNOS expression in astrospheres.* Excessive nitric oxide production by iNOS participates in the pathogenesis of multiple sclerosis and there are grounds for assuming that NOS inhibitors may play a positive role in the treatment of the above-mentioned neurodegenerative diseases.

Inducible NOS makes a substantial contribution to the development of chronic inflammations. At the peak of a chronic inflammation, an eightfold increase in the NOS activity is observed, of which more than 90% falls to iNOS. The use of selective iNOS inhibitors is favorable for the therapy. Induced arthrites in rats are exacerbated upon administration of L-arginine and are relieved by iNOS inhibitors. In the case of rheumatoid arthritis, increased concentrations of nitrites and nitrates can be found in the synovial fluid* of patients having increased local release of nitric oxide.[3]

Psoriatic skin damages contain a high level of iNOS, which is missing from healthy skin; the expression of nNOS is also enhanced during psoriasis. This higher level of enzymes increases nitric oxide production on the skin surface by an order of magnitude. This indicates that NO plays a significant role in the development and (or) maintenance of psoriasis diseases, and the use of NOS antagonists seems a promising line in the therapy of these diseases. These inhibitors can also be used successfully to treat asthma or intestinal diseases. Although the problem of stopping inflammatory pain syndromes by NOS inhibitors is not entirely clear, this approach appears practicable, especially in view of the fact that 7-nitroindazole is active *in vivo* as an anaesthetic.

It is known[3] that nitroglycerin (NG) causes migraine, headache being heavier upon its combination with *N*-acetylcysteine (the latter potentiates the NG action on the cardiovascular system). Nitroglycerin causes headache in patients suffering from migraine attacks more often than in healthy people. NOS inhibitors, for example, L-NMMA, are efficient for removing such states. This proves that nitric oxide participates in the migraine development. Serotonin (**108**) can also be involved in migraine initiation and maintenance, although the mechanism of its action is still unclear. The agonists of serotonin receptors, for example, sumatriptan (**109**), are efficient in later stages.



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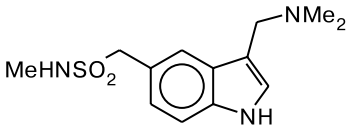

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A hypothesis was put forward[3] according to which migraine is caused by the release of serotonin in brain vessels; the action of this central nervous system mediator on the serotonin 5-$HT_{2B/2C}$ receptors (subspecies of sero-

* Cells acting as the supporting structures of the nerve tissue.

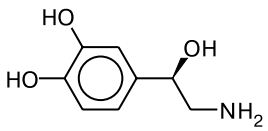
* The liquid in the joint cavity functioning as the lubricant.

tonin receptors) results in the release of nitric oxide from endothelium. The formation of NO induces vasodilation, receptor activation of nerve fibers, and an increase in sensitivity; this gives rise to an inflammatory-like process characterized as migraine.

The insulin-dependent pancreatic diabetes is an autoimmune disease,* leading to the destruction of pancreatic β-cells (insulin-secreting cells). One of the mechanisms by which cytokines inducing this process (interleukin-1β is especially active in this respect)** destroy β-cells is iNOS induction inside the cells.[84] The generation of nitric oxide inactivates β-cell enzymes containing transition metal ions, which sharply decreases the insulin-secreting capacity of the cells. Therefore, iNOS inhibition is now regarded as a promising way of treatment of insulin-dependent diabetes.

Lipopolysaccharides (endotoxins) of gram-negative bacteria increase the permeability of the blood—brain barrier (BBB), which results in brain edema and violates the cerebral blood flow and metabiolic processes in brain. This is followed by neuronal dysfunctions, neurotoxicity, *i.e.*, phenomena defined as inflammatory meningitis. Studies along this line are usually focused on the role of factors that cause the inflammation such as prostaglandins, thromboxanes, cytokines, active forms of oxygen, *etc*. Currently, the problem of nitric oxide involvement in the pathogenesis of meningitis is under investigation.[3] In this respect, various selective NO inhibitors have been studied. It was shown that L-nitroarginine (L-NNA) decreases the intensity of brain edema, normalizes the intracranial pressure and the nitrite level in the cerebrospinal fluid. Aminoguanidine (selective iNOS inhibitor) prevents the increase in BBB permeability in experimental meningitis and decreases the excessive production of nitric oxide. This altogether implies that NO is an important inflammatory mediator involved in the pathogenesis of the bacterial meningitis; thus, NO synthase antagonists may be favorable in the therapy of these diseases.

An important problem concerns the relationship of NO with other nervous system mediators. The vascular tone and the local blood flow are regulated by a number of systems, the sympathetic nervous system and the systems related to nitric oxide synthesis being the most important among them. The sympathetic nervous system is mediated by (*R*)-noradrenaline (**110**).



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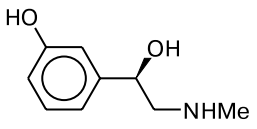


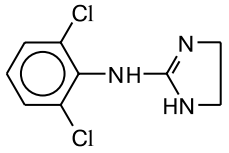
111

* The response to autoimmune antigens, *i.e.*, organism own antigens or antigens arising in response to an external action with respect to which autoantibodies are formed.

** See Section 1, Footnote on p. 1974.

In addition, neuropeptide Y (NPY)* acts as a comediator (see Ref. 85). Presumably,[86] nitric oxide release entails a loss of vessel responsiveness to adrenergic stimulation during sepsis and NO inhibits adrenergic vasoconstriction. A study showed[86] that endogenous NO is a modulator of the vasoconstrictory effect of phenylephrine (**111**), which is an agonist of the adrenergic system, and NPY.

It was shown[86] that NO donors (for example, sodium nitroprusside) weaken, and NOS inhibitors such as L-NAME enhance the pressor effect of noradrenaline. Interestingly, the possibility of NO release upon administration of noradrenaline was pointed out.[86] It is known that the activation of α_2-adrenergic receptors located mainly in endothelium** may induce NO release.[87—89] Meanwhile, no exact data on the influence of activation of α_1-adrenergic receptors on the formation of nitric oxide are currently available, but in the opinion of the researchers cited,[86] this cannot be ruled out. The relationship found between α_2-adrenergic receptors and NO is of particular interest due to the fact that both a classical stimulator of these receptors, clonidine (**112**), and an agonist of imidazoline and α_2-adrenergic receptors, moxonidine (**113**), are nitric oxide donors.[2] The relationship between the adrenergic system and NO in a living organism may prove closer than it is now considered.



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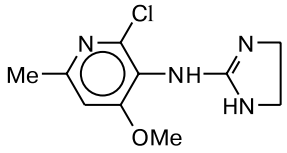

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It was found[86] that, in addition to modulation of the adrenergic and NPY mechanisms, NO inhibits the vasoconstrictory effect of angiotensin AT_1 [90,91] (the role of peptides angiotensin I and angiotensin II in maintenance of blood pressure is described in more detail in Refs. 13—15).

In a recent study,[92] it was found that NO produced by the inducible NO synthase is responsible, together with peroxynitrite (see Ref. 2) for the damaging effects of endotoxins on cell functions and that iNOS activation may be due to liver and myocardium dysfunctions caused by endotoxic shock. This clarifies the role of selective iNOS inhibitors the search for which is currently in progress.

Recently, a study has been published[93] dealing with the role of nitric oxide in the case of thermal injuries. The

* NPY is a neuropeptide common in the central nervous system and in the peripheral nervous system; it comprises 36 amino acids, exhibits anxiolytic (anti-anxiety, reducing the obsessive fear) and sedative effects and vasoconstriction properties.

** A thin layer of flat cells lining the cardiac cavity and blood and lymphatic vessels.

tissue hyperpermeability caused by burns and numerous inflammatory processes arising thereupon can be regulated by pharmacotherapy at early stages. It is also possible to prevent the subsequent systemic hyperpermeability. The inflammatory responses include, in particular, the release of nitric oxide. It was shown[94] that the NO concentration both near the burn and in the blood plasma increases and that NOS inhibitors suppress the vessel hyperpermeability in the wound area of the injury. It is well-known that the administration of NOS inhibitors entails an increase in blood pressure. Long-term administration of L-NAME induces hypertension and left ventricular hypertrophy. This implies that vessel hyperpermeability (typical of their thermal injury) at the burn site (but not in the unaffected tissue) is suppressed by administrating NO synthase inhibitors. Presumably, nitric oxide level at the burn site increases in response to inflammatory processes and these processes can be reduced by NOS antagonists. It was shown experimentally that constitutive NOS inhibitors are useful at early, while inducible NOS inhibitors, at later stages of a thermal injury.

8. Conclusion

This review should be regarded as a continuation of the previous review[2] devoted to the problems of generation of both endogenous and exogenous nitric oxide in the organism. It is clear that discussion of the approaches to increasing (if necessary) the amount of NO in organs and tissues cannot provide a full picture without similar consideration of the ways of decreasing its concentration in the case of excess release of NO at various pathological states. It should be stressed once again that nowadays it is impossible to cover, within the framework of journal articles, the enormous and ever increasing body of information concerning various aspects of the biological action of nitric oxide and the chemical investigations aimed ultimately at the search for new, efficient drugs that act selectively depending on insufficient or excessive NO production. For this reason, both here and in the previous reviews, we generalize recent (as a rule, published after 1994) studies that we consider fundamentally important and having the most crucial influence on the development of medicinal chemistry, biology, and biochemistry. Of course, it should be borne in mind that these reviews have been written by chemists and meant for chemists. Therefore, many specific medical and biological problems are discussed only cursory or totally left off-screen.

Finally, we would like to say a few words about the general problem of NO functioning in a living organism. When a new paradigm, a new conceptual scheme, or a new model for problem definition and solution appear in science, the impression always arises that this would solve numerous problems that could not be understood before. It is beyond doubt that the brilliant discoveries concerning the enormous role played by nitric oxide in a living organism ensured a real breakthrough in biology and medicinal chemistry by initiating new lines of research in these fields of science.

However, at present, it should be noted that the original euphoria has started to go down. Simple solutions that seemed to suggest themselves on the basis of the initial data gained in the studies of NO donors and NOS inhibitors proved to be, at least, incomplete and inadequate in many respects. In the reviews, we endeavored to pay attention to vague and contradictory items, which are more and more frequently discussed in the literature.

Nevertheless, one cannot but emphasize that less than 15 years have passed since it became clear that the mysterious compound, the endothelium-derived relaxing factor (EDRF) is an unstable, toxic gas constantly released in the organism of mammals (or a form of its deposition) whose role in vital processes cannot be overestimated.

We would like to note that investigation of many known drugs provides grounds for asserting that some of them are nitric oxide donors.[2] In this connection, the influence of the considered processes on the mechanism of action of drugs used for a long time in medical practice should be thoroughly examined. In addition, the possibility of looking for nitric oxide synthase inhibitors (perhaps, selective inhibitors) among known drugs has not been seriously surveyed either. Meanwhile, it is beyond doubt that this problem deserves meticulous attention, which could result in considerable progress in both theoretical and practical respects.

References

1. V. G. Granik, S. Yu. Ryabova, and N. B. Grigor´ev, *Usp. Khim.*, 1997, **66**, 792 [*Russ. Chem. Rev.*, 1997, **66** (Engl. Transl.)].
2. V. G. Granik, and N. B. Grigor´ev, *Izv. Akad. Nauk, Ser. Khim.*, 2002, **51**, 1268 [*Russ. Chem. Bull., Int. Ed.*, 2002, **51**, 1375].
3. A. J. Hobbs, A. Higgs, and S. Moncada, *Annu. Rev. Pharmacol. Toxicol.*, 1999, **39**, 191.
4. A. R. Seminara, J. S. Krumenasker, and F. Murad, in *Nitric Oxide, Basic Research and Clinical Applications*, Eds. R. J. Gryglewski and P. Minuz, IOS Press, Amsterdam—Berlin—Oxford—Tokyo—Washington, DC, 2000, 5.
5. J. M. Fukuto and G. Chaudhuri, *Annu. Rev. Pharmacol. Toxicol.*, 1995, **35**, 165.
6. M. D. Mashkovskii, *Lekarstvennye sredstva*, *Posobie dlya vrachei* [*Drugs. Doctors´ Textbook*], issue 13, Khar´kov, Torgsin, 1997, **1**, 543 pp.; **2**, 592 pp. (in Russian).
7. M. D. Mashkovskii, *Lekarstva XX veka* [*XX Century Drugs*], Novaya volna, Moscow, 1998, 319 pp. (in Russian).
8. R. B. Barlow, *Introduction to Chemical Pharmacology*, J. Wiley and Sons, London—New York, 1954.

9. A. L. Lehninger, *Principles of Biochemistry*, Worth Publishers, Inc., New York, 1982.
10. D. A. Kharkevich, *Farmakologiya* [*Pharmacology*], Moscow, Meditsina, 1999, 661 pp. (in Russian).
11. A. Albert, *Selective Toxicity. Physico-Chemical Basis of Therapy,* Chapman and Hall, London—New York, 1985.
12. J. G. Cannon, *Pharmacology for Chemists*, Oxford University Press, New York, Oxford, 1999, 272 p.
13. G. I. Zhungietu and V. G. Granik, *Osnovnye printsipy konstruirovaniya lekarstv* [*Main Principles of Drug Design*], IPK Gos. univ. Moldovy, Kishinev, 2000, 300 pp.
14. V. G. Granik, *Lekarstva, farmakologicheskii, biokhimicheskii i khimicheskii aspekty* [*Drugs. Pharmacological, Biochemical, and Chemical Aspects*]. Vuzovskaya kniga, Moscow, 2001, 408 pp. (in Russian).
15. V. G. Granik, *Osnovy meditsinskoi khimii* [*Foundations of Medicinal Chemistry*],Vuzovskaya kniga, Moscow, 2001, 384 pp. (in Russian).
16. *Nitric Oxide: Brain and Immune System*, Eds. S. Moncada, G. Nistico, and E. A. Higgs, Portland Press, London—Chapel Hill, 1993, 293 p.
17. S. Moncada and A. Higgs, *FASEB J.*, 1995, **9**, 1315,
18. O. W. Griffith and S. S. Gross, *Methods in Nitric Oxide Research*, Eds. M. Feelish and J. S. Stamler, Wiley, Chichester, 1996, p. 187.
19. J. T. Groves and C. C.-Y. Wang, *Current Opinion Chem. Biol.*, 2000, **4**, 687.
20. Y. Su, M. Couch and E. R. Block, *Nitric Oxide: Biology and Chemistry*, 1997, **1**, 469.
21. S. Pfeifer, E. Leopold, K. Schmidt, F. Brunner, and B. Mayer, *Br. J. Pharmacol.*, 1996, **118**, 1433.
22. N. M. Olken and M. A. Marletta, *Biochemistry*, 1993, **32**, 9677.
23. N. M. Olken, Y. Osawa, and M. A. Marletta, *Biochemistry*, 1994, **33**, 14784.
24. P. Klatt, K. Shmidt, F. Brunner, and B. Mayer, *J. Biol. Chem.*, 1994, **269**, 1674.
24. O. W. Griffith and D. J. Stuehr, *Annu. Rev. Physiol.*, 1995, **57**, 707.
26. K. Narayanan, L. Spack, M. Hayward, and O. W. Griffith, *FASEB J.*, 1994, **8**, A360.
27. N. M. Olken and M. A. Marletta, *J. Med. Chem.*, 1992, **35**, 1137.
28. J.-Mi Hah, L. J. Roman, and R. B. Silverman, *Bioorg. Med. Chem.*, 2000, **8**, 1931.
29. M.-X. Wang, D. F. Murrell, C. Szabo, R. F. Warren, M. Sarris, and G. A. C. Murrell, *Nitric Oxide: Biology and Chemistry*, 2001, **5**, 219.
30. G. Marechal and P. Gailly, *Cell Mol. Life Sci.*, 1999, **55**, 1088.
31. S. Pfeiffer, E. Leopold, K. Schmidt, F. Brunner, and B. Mayer, *Br. J. Pharmacol.*, 1996, **118**, 1433.
32. S. A. Lipton, N. J. Sucher, P. K. Kaiser, and E. B. Dreyer, *Neuron*, 1991, **7**, 111.
33. S. Moncada, R. H. J. Palmer, and E. A. Higgs, *Pharmacol. Rev.*, 1991, **43**, 109.
34. M. A. Marletta, *J. Biol. Chem.*, 1993, **268**, 1223.
34. J. F. Kerwin and M. Heller, *Med. Res. Rev.*, 1994, **14**, 23.
36. J. L. Collins, J. A. Oplinger, S. Lee, E. P. Garvey, M. Sulter, C. Duffy, T. C. Burnette, and E. S. Furfine, *J. Med. Chem.*, 1998, **41**, 2858.
37. P. L. Feldman, O. W. Griffith, H. Hong, and D. J. Stuehr, *J. Med. Chem.*, 1993, **36**, 491.
38. S. Cellek and S. Moncada, *Nitric Oxide: Biology and Chemistry*, 1997, **1**, 204.
39. J.-Mi Hah, L. J. Roman, P. Martasek, and R. B. Silverman, *J. Med. Chem.*, 2001, **44**, 2667.
40. H. Huang, P. Martasek, L. J. Roman, R. S. S. Masters, and R. B. Silverman, *J. Med. Chem.*, 1999, **42**, 3147.
41. H. Huang, P. Martasek, L. J. Roman, and R. B. Silverman, *J. Med. Chem.*, 2000, **43**, 2938.
42. *Annual Drug Data Rep.*,* 1996, **18**, (a) 681; (b) 144; (c) 1017; (d) 527; (e) 786; (f) 785; (g) 887; (h) 873; (i) 697; (j) 967.
43. *Annual Drug Data Rep.*, 1995, **17**, (a) 257; (b) 535; (c) 555; (d) 914; (e) 135; (f) 565; (g) 707; (h) 820; (i) 530; (j) 444; (k) 707.
44. *Annual Drug Data Rep.*, 1998, **20**, (a) 45; (b) 210; (c) 46; (d) 134; (e) 301; (f) 404; (g) 706; (h) 978; (i) 935; (j) 160; (k) 934; (l) 310; (m) 403; (n) 669; (o) 29; (p) 404.
45. J. Prous, T. Wroblewski, and T. Gastacer, *Drug Future*, 1998, **23**, No. 2, 123.
46. K. Narayanan, L. Spack, M. Hayward, and O. W. Griffith, *FASEB J.*, 1994, **8**, A360.
47. K. Naraynan and O. W. Griffith, *J. Med. Chem.*, 1994, **37**, 885.
48. E. S. Furfine, M. F. Harmon, J. E. Paith, R. G. Knowles, M. Salter, R. J. Kiff, C. Duffy, R. Haselwood, J. A. Oplinger, and E. P. Garvey, *J. Biol. Chem.*, 1994, **269**, 26677.
49. W. N. Moore, R. K. Weber, G. M. Jerome, F. S. Tjoeng, T. P. Misco, and M. G. Currie, *J. Med. Chem.*, 1994, **37**, 3886.
50. *Annual Drug Data Rep.*, 1999, **21**, (a) 663; (b) 320; (c) 976; (d) 528; (e) 587; (f) 967; (g) 28; (h) 123; (i) 124; (j) 966; (k) 392; (l) 495; (m) 663.
51. J. Ou, T. M. Carlos, S. C. Watkins, J. E. Saavedra, J. K. Keefer, Y.-M. Kim, R. G. Harbrecht, and T. R. Billear, *Nitric Oxide: Biology and Chemistry*, 1997, **1**, 404.
52. *Annual Drug Data Rep.*, 1994, **16**, (a) 731; (b) 459; (c) 923; (d) 219.
53. *Annual Drug Data Rep.*, 2000, **22**, (a) 177; (b) 199; (c) 775; (d) 132; (e) 147; (f) 999.
54. Y. Lee, P. Martasek, L. J. Roman, B. S. Siler Master, and R. B. Silverman, *Bioorg. Med. Chem.*, 1999, **7**, 1941.
55. J. Prous, A. Graul, and J. Castacer, *Drug Future*, 1994, **19**, 740.
56. T. Tabatabaie, K. L. Graham, A. M. Vasques, R. A. Floyd, and Y. Katake, *Nitric Oxide: Biology and Chemistry*, 2000, **4**, 157.
57. H. Ruetten and C. Thiemermann, *Br. J. Pharmacol.*, 1996, **119**, 479.
58. *Annual Drug Data Rep.*, 1997, **19**, (a) 548; (b) 77; (c) 328; (d) 622; (e) 737; (f) 701; (g) 236; (h) 549; (i) 217; (j) 43.
59. G. L. Southan, C. Szabo, M. P. O´Connor, A. L. Salzman, and C. Thiemermann, *Europ. J. Pharmacol.*, 1995, **291**, 311.
60. G. L. Southan, C. Szabo, and C. Thiemermann, *Br. J. Pharmacol.*, 1995, **114**, 510.
61. C. Szabo, G. L. Southan, and C. Thiemermann, *Proc. Natl. Acad. Sci. USA*, 1994, **91**, 12472.

* *Annual Drug Data Report series*, Ed. J. R. Prous, Prous Science, S.A., Barcelona—Philadelphia.

62. L. H. Wei, N. Arabolos, and L. J. Ignarro, *Nitric Oxide: Biology and Chemistry*, 1998, **2**, 155.
63. S. Ulhag, E. C. Chinje, M. A. Naylor, M. Jaffer, J. J. Stafford, and M. D. Threadgill, *Bioorg. Med. Chem.*, 1999, **7**, 1941.
64. D. W. Chow, *Proc. Natl. Acad. Sci. USA*, 1993, **90**, 9741.
65. T. M. Dowson, V. L. Dowson, and S. H. Snyder, *Ann. Neurol.*, 1992, **32**, 297
66. E. P. Garvey, J. V. Title, R. K. Covinton, B. H. Merrill, E. R. Wood, S. A. Baylis, and S. A. Charles, *Arch. Biochem. Biophys.*, 1994, **311**, 235.
67. E. P. Garvey, J. A. Oplinger, E. S. Furfine, R. J. Kiff, F. Laszlo, B. J. R. Whittle, and R. C. Knowles, *J. Biol. Chem.*, 1997, **272**, 4959.
68. F. N. Fastier, *Pharmacol. Rev.*, 1962, **14**, 37.
69. D. J. Wolf, G. A. Datto, A. Samatovicz, and R. A. Tempsick, *J. Biol. Chem.*, 1993, **268**, 9425.
70. D. J. Wolf, A. Libesski, and S. C. Umansky, *Arch. Biochem. Biophys.*, 1994, **311**, 360.
71. D. J. Wolf and B. J. Gribin, *Arch. Biochem. Biophys.*, 1994, **311**, 293.
72. R. L. C. Handy and P. K. Moore, *Life Sci.*, 1997, **60**, 389.
73. V. Sorrenti, C. D. Giacomo, L. Salerno, M. A. Siracusa, F. Guerrera, and A. Vanella, *Nitric Oxide: Biology and Chemistry*, 2001, **5**, 32.
74. D. I. Metelitsa, *Aktivatsiya kisloroda fermentnymi sistemami* [*Oxygen Activation by Enzyme Systems*], Nauka, Moscow, 1982, 255 pp. (in Russian).
75. J. M. Quintella, C. Peinador, L. M. Gonzalez, R. Riguera, I. Rioja, M. C. Terencio, A. Ubeda, and M. J. Alkarez, *J. Med. Chem.*, 1999, **42**, 4720
76. G. Yue, F. F. Sun, C. Dunn, K. Yin, and P. Y. K. Wong, *J. Pharmacol. Exp. Theor.*, 1996, **276**, 265.
77. T. Honda, B. V. Rounds, F. G. Favaloro, G. W. Gribble, N. Suh, X. Wang, and M. B. Sporn, *Bioorg. Med. Chem. Lett.*, 1999, **9**, 3429.
78. Y.-X. Wang, C. I. Poon, K. S. Poon, and C. C. Y. Pang, *Br. J. Pharmacol.*, 1993, **110**, 1232.
79. C. Szabo, *New Horiz.*, 1995, **3**, 2.
80. B. S. Teilor, L. Kh. Alarson, and T. R. Billiar, *Biokhimiya*, 1998, **63**, 905 [*Biochemistry (Moscow)*, 1998, **63** (Engl. Transl.)].
81. L. Laudet, A. Rosselet, M. D. Schaller, M. Markert, C. Perret, and F. Feihl, *J. Infect. Dis.*, 1998, **177**, 127.
82. S. Bergamini, C. Rota, R. Canali, M. Staffieri, F. Daneri, A. Bini, F. Giovannini, A. Tomasi, and A. Jannone, *Nitric Oxide: Biology and Chemistry*, 2001, **5**, 349.
83. F. Squadrito, D. Altavilla, G. Squadritta, G. M. Campo, M. Ioculano, P. Canale, F. Rossi, A. Saitta, and A. P. Caputi, *Br. J. Pharmacol.*, 1996, **119**, 23.
84. J. A. Corbett, G. Kwon, T. P. Mesko, C. P. Rody, and M. L. McDunuel, *Am. J. Physiol.*, 1994, **267**, 48.
85. R. E. Malmstrom, *Acta. Physiol. Scand.*, 1997, **160**, Suppl. 636, 1.
86. R. E. Malmstrom, H. Bjorne, K. Alving, E. Weitzberg, and J. O. N. Lundberg, *Nitric Oxide: Biology and Chemistry*, 2001, **5**, 98.
87. M. R. Hynes, H. Dong, and S. P. Duckles, *Life Sci.*, 1987, **42**, 357.
88. T. M. Cocks and J. A. Angus, *Nature*, 1983, **305**, 627.
89. C. Egleme, T. Godfraind, and R. C. Miller, *Br. J. Pharmacol.*, 1984, **81**, 16.
90. K. P. Konrad and S. L. Whittemore, *Am. J. Physiol.*, 1992, **262**, R1137.
91. S. Ito, C. S. Johnston, and O. A. Carretero, *J. Clin. Invest.*, 1991, **87**, 1656.
92. J.-C. Preiser, H. Zhang, B. Vray, A. Hrabak, and J.-L. Vincent, *Nitric Oxide: Biology and Chemistry*, 2001, **5**, 208.
93. H. Inoue, K. Ando, N. Wakisaka, K.-I. Matsuzaki, M. Aihara, and N. Kumagai, *Nitric Oxide: Biology and Chemistry*, 2001, **5**, 334.
94. T. Sozumi, *Ann. Plast. Surg.*, 1997, **39**, 272.

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