Reviews

Biotransformations of drugs belonging to nitrogen heterocycles

V. G. Granik

Center for Drug Chemistry,
7 stroenie, 1 ul. Zubovskaya, 119021 Moscow, Russian Federation.
Tel.: +7 (499) 246 9384. E-mail: vggranik@mail.ru

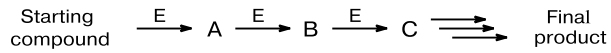
The present review covers general aspects related to the metabolism of organic compounds, primarily, of biologically active azaheterocycles used as drugs. Compounds of this type occupy a dominant place among known drugs. Data on the main chemical processes determining the pathways of principal biotransformations, such as redox reactions, hydrolysis, alkylation, acylation, various conjugations, isomerizations, and condensations, are summarized. The metabolisms of heterocycles is considered based on the ring size of the substrate.

Key words: cell metabolism, biotransformation, azaheterocycles, oxidation, reduction, enzymatic transformations, hydrolysis, alkylation, acylation, conjugations, isomerizations, condensations.

General problems

A huge number of processes that occur in living cells are collectively called metabolism.[1—4] In the most commonly used meaning, the term "metabolism" is equal to the term "material and energy exchange." In the present review, reactions involved in the metabolism of particular drugs (belonging primarily to nitrogen-containing heterocycles) in living organisms are considered in detail. Let us start with the consideration of some general problems related to the functions and stages of metabolism, such as the digestion of nutrients and the degradation of macromolecules and other organic compounds. The cell metabolism is a system of enzymatic transformations not only of substances but also of the energy, resulting in the biosynthesis of the living matter. The products of successive transformations involved in the metabolic path can be represented by Scheme 1.

Scheme 1



E is the enzyme

Products A, B, and C and the final product are called metabolites.

The functions of metabolism are as follows:

1) the conversion of the chemical energy into other types of energy to perform various transformations of sub-

Published in Russian in *Izvestiya Akademii Nauk. Seriya Khimicheskaya,* No. 1, pp. 1—36, January, 2010.
1066-5285/10/5901-0001

stances that are taken into the body with food or the conversion of solar energy;

2) the transformation of nutrients into the starting compounds for the construction of macromolecules;

3) the assembly of proteins, polysaccharides, lipids, nucleic acids, and other macromolecules;

4) the synthesis and degradation of biomolecules performing particular functions in cells.

Let us consider two main stages of metabolism comprising the overall process:

1) the catabolism is the phase, which leads to the breakdown of complex organic molecules (carbohydrates, fats, and proteins are finally degraded to carbon dioxide, ammonia, and water) and which is accompanied by a release of the free energy (this stage results in the formation of hydrophilic compounds or their conjugates, which can be rapidly removed from the body);

2) the anabolism or biosynthesis is the synthetic phase, during which small building blocks are assembled into macromolecules accompanied by the consumption of energy from such sources as adenosine triphosphate (ATP), adenosine diphosphate (ADP), inorganic phosphates (Pi), nicotinamide adenine dinucleotide phosphate (NADP), *etc.*

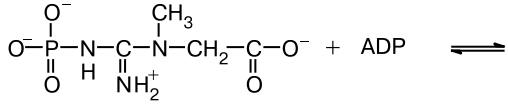
The anabolic stages (exemplified by the protein synthesis) involve the formation of α-keto acids and the construction of peptide compounds. The catabolic and anabolic pathways oppose each other but share the stage involving the citric acid cycle (Krebs cycle) and a number of other auxiliary enzymatic reactions. Adenosine triphosphate is the major chemical link between cellular reactions accompanied by the release or consumption of energy. The "cellular fuel" produced in different enzymatic processes is cleaved, and a part of the free energy that is released is used to drive the synthesis of ATP from ADP and Pi. In other phases of metabolism, ATP loses the phosphate fragment to form ADP, and the released energy is consumed in driving other processes.

Therefore, ATP transports chemical energy and provides a universal association between different cellular processes accompanied by the release or consumption of energy. The energy released from the breakdown of ATP is consumed for different purposes enabling the biosynthesis (chemical work), the active transport across membranes (conversion of the chemical energy into the osmotic work), and the muscle contraction (conversion of the chemical energy into the mechanical energy).

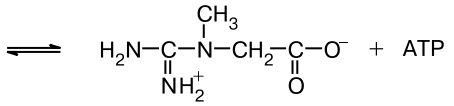
The energy released during catabolism is conserved in phosphorylated compounds, and the transfer of the latter to ADP catalyzed by specific kinases gives ATP. When needed, another specific kinase transfers the phosphate group of ATP to a molecules serving as a phosphate acceptor, thus increasing the energy of this molecule and enabling successive chemical (or other) processes.

The muscle releases (like the muscle contraction) is associated with the hydrolysis of ATP to ADP and inorganic phosphates and the energy release. Another type of the work performed by cells is associated with the conversion of the energy of ATP into the osmotic work, *viz.*, the transport of a substance against the concentration gradient (active transport). An example is the so-called parietal cells maintaining the maximum concentration gradient. These are the cells of the mucous coat of stomach, which secrete hydrochloric acid. The concentration of HCl in gastric juice is as high as 0.1 mol L^{-1} (pH 1), whereas the concentration of HCl in surrounding cells is 10^{-7} mol L^{-1} (pH 7), *i.e.*, parietal cells secrete H^+ against the gradient of about 10^6 : 1. Presumably, these cells are equipped with membrane pumps for the secretion of hydrochloric acid. The formation of gastric HCl is stimulated by the special enzyme H^+-transporting ATPase, which also catalyzes the degradation of ATP to ADP accompanied by the energy release. The sodium and potassium ion transport across plasma membranes of living cells is another example of the active transport following the same principle. The consumption of ATP supporting the energy needs of cells should be continuously renewed. In particular, the ATP concentration in muscles is maintained at a rather high level due to the presence of a considerable amount of creatine phosphate (**1**), which serves as a reservoir of high-energy phosphate groups in muscles due to the reversible reaction with ADP that yields creatine (**2**) and ATP (Scheme 2).

Scheme 2



1



2

When ATP is partially consumed to perform a particular work, for example, for the muscle contraction under the action of creatine kinase, another reaction always returns the amount of adenosine phosphate in cells to the normal level.

The present review considers, in addition to the general aspects, the biotransformation of some drugs in living organisms. Taking into account the main aim of the review, let us start with the consideration of the problems related to the biotransformation of xenobiotics (predominantly belonging to the azaheterocyclic series). By xenobiotics are meant compounds, which are not involved in such functions of the body, as the energy production and many other physiologically important processes.

An important point, which calls for special discussion, is the ambiguity of the processes involved in the metabolism of xenobiotics and the consequences dictated by this ambiguity. It is necessary to consider the nature of substances formed by degradation, their useful and potentially harmful properties, their pharmacological activity, toxicological properties, and methods and completeness of their removal from the body. Hence, it is essential to first discuss the results of principal pharmacokinetic studies and problems concerning the predominant pathways of metabolic processes. First, let us consider the major chemical processes determining the pathways of metabolic transformations. These are redox processes, hydrolysis, hydration, and various alkylation (primarily methylation), acylation, conjugation, condensation, and isomerization reactions.

In the first phase of these processes, substances are rendered (by the introduction of the corresponding functional groups) accessible for the second phase, *viz.*, the detoxification and the formation of products, which can be eliminated from the body. It is very important that enzymatic systems specific for interactions with endogenous compounds are often also involved in the degradation of xenobiotics. Apparently, this fact should be considered as a result of a certain structural (or electronic) similarity between these and other types of substances, which is not always evident from a comparison of the chemical formulas of these compounds. Apparently, during evolution living organisms have evolved the ability to use the enzymes synthesized in the organism for the catalysis of a huge number of reactions associated not only with biotransformations of endogenous compounds but also with the metabolism of various xenobiotics, which are often structurally very different from the former compounds. It is still difficult to decide which factor, *viz.*, the suitability for the catalysis of the first type of reactions or for the processes related to foreign substances, plays the primary role. However, based on the general considerations and available experimental data, it can be stated that the enzymes involved in metabolic transformations of xenobiotics do not have high substrate specificity because of the high structural variability of compounds. The catalysis by these enzymes often provides a relatively low reaction rate. It is important that these enzymes are specific for lipophilic substrates and convert the latter into more polar molecules, because the main function of metabolism of foreign substances is to enable their removal from the body.

Apparently, when such analogies can be revealed, they can be useful in finding new original drugs. It seems that the question about the similarity of the enzymes, which can be involved in metabolic processes aimed at the degradation of endogenous and exogenous compounds, is the determining factor for the choice of the structures of particular interest for pharmacological studies.

Before turning to the more detailed discussion of the above-mentioned phases of metabolism, let us mention yet another important factor, which is also promising for the drug design. Thus, it is necessary to create conditions, under which substances that can act directly or indirectly on a particular receptor system (either on enzymes or protein structures) are delivered to organs or tissues, where these interactions are effective.

The substance transport across plasma membranes is of fundamental importance for all living cells. The mechanisms of the transport of small molecules across plasma membranes can be divided into three types: the diffusion, the facilitated diffusion, and the active transport.

The diffusion is the process by which molecules or ions are transported across membranes from the high-concentration region to the low-concentration region *via* the Brownian motion. This transport occurs until the concentrations become equal. Hydrophobic compounds diffuse across lipid membranes more rapdily than hydrophilic compounds. The transport of neutral molecules occurs *via* neutral diffusion, whose rate decreases with increasing hydrophilicity of the molecules. The ion diffusion is the process by which the charged ions are transported across membranes. This is the passive process, which also depends on the transmembrane concentration difference.

The facilitated diffusion is the process by which molecules are transported from the high-concentration region to the low-concentration region, the transport being mediated by carrier proteins located in plasma membranes. This process is passive in the sense that the transport occurs along the concentration gradient and is characterized by the following features:

1) it is specific for particular molecules;

2) it occurs more rapidly than the usual diffusion;

3) it reaches the saturation.

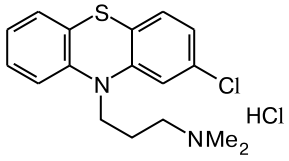
Specific carriers are available for many molecules, such as glucose, lactose, amino acids, nucleotides, glycerol, *etc*. The selectivity is determined by the presence of the stereospecific binding site in the carriers (for example, the glucose carrier performs the transport of only D-glucose but not of L-glucose). In the latter case, the binding of this molecule to the carrier is followed by the structural transformation as a result of which the small molecule is transported to the other side of the membrane. In this case, the transport rate across the plasma membrane is substantially higher than the rate of simple diffusion. Apparently, this mechanism occurs during evolution to enable the transport of such hydrophilic molecules, which would otherwise diffuse across membranes too slowly to meet the needs of cells. In any cell, there is a certain number of carriers for this particular molecule or ion, and when all these carriers are occupied, the transport rate decreases. Consequently, the process reaches the saturation. Some hormones, such as insulin and the epidermal growth factor,

can cause an increase in the rate of the carrier-mediated diffusion compared to that of the usual diffusion.

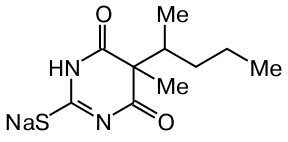
The active transport is the process by which ions or molecules are transported across cell membranes against the concentration gradient, which requires external energy. Sodium potassium ATPase is one of the most well studied active transport systems. In most cells, different concentrations of Na^+ and K^+ ions are maintained inside and outside the cells. The concentration ratio for each ion can be as high as 10—15. Generally, the concentration of K^+ ions inside cells is high, whereas the concentration of Na^+ ions is low and *vice versa* outside the cells. Since membranes are, though weakly, permeable to charged ions, there is a slight leakage, and the concentrations of these two types of ions on the opposite sides of the membranes tend to be equal. Sodium potassium ATPase assists in the active ion transport to prevent this equalization.

The brain penetration of drugs (and xenobiotics) deserves special consideration. The diffusion into the brain is more hindered compared to that into other tissues of the body. The hematoencephalic barrier (HEB) protecting the brain from foreign substances is composed of high-density cells restricting passage and, though being permeable to lipophilic substances, it is highly impermeable to ions. The situation is different in inflammatory processes, and many substances can cross the hematoencephalic barrier. Once the latter occurs, the substance can penetrate through membranes within the brain followed by the distribution over different areas of the brain.

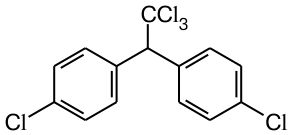
Highly lipophilic compounds, such as Aminazine, Thiopental, and DDT, are found in the gray substance even after the peroral administration, and are accumulated with time in the white substance of the brain.



Aminazine



Thiopental



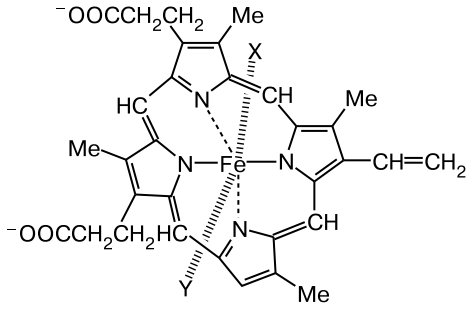
DDT

The elimination of xenobiotics can occur in different ways: the elimination of the substance from the body in the unchanged form, the elimination of substances produced by non-enzymatic transformations, and, finally, the elimination of substances, which are formed through the enzyme-catalyzed metabolism. The metabolism in the body occurs primarily with the involvement of enzymes.

The urinary excretion, which involves the glomerular filtration, the passive reabsorption, and the active tubular transport, is among the most important excretion pathways. The kidney excretion of chemical compounds decreases with increasing degree of binding to proteins and with increasing lipophilicity due to the enhancement of tubular reabsorption. The fecal excretion is yet another type of elimination. This process is determined by the reabsorption of substances in the gastrointestinal tract, the bile excretion, or the passive diffusion from the blood into the intestinal lumen. The eliminations through the skin and lungs are also essential pathways.

Let us revert to the phases of metabolism. Reactions catalyzed by cytochrome P450 (cytochromes are microsomal mixed-function oxidases) belong to very important processes occurring in the first phase. Cytochrome P450 is the hemoprotein found in all living organisms, such as bacteria, yeast, and animals (insects and vertebrates). In mammalians, cytochromes P450 were found in all organs and tissues. These membrane-bound enzymes are located primarily in the endoplasmatic reticulum. Cytochromes P450 are present also in mitochondria and almost in all membranes and cells. The chemical structure of cytochrome P450 consists of the protein part and the heme moiety, *viz.*, iron protoporphyrin-IX.

This porphyrin is involved also in other hemoproteins and enzymes, such as hemoglobin, myoglobin, catalase, and most of peroxidases.



Iron protoporphyrin-IX

The fifth ligand at the iron cation (X) is the thiolate anion of cysteine located in the vicinity of the C terminus of the protein. The strong iron—sulfur bond is responsible for a relatively high electron density on iron. The sixth ligand site (Y) is most likely occupied by the hydroxyl group of the nearby amino acid residue or a water molecule. In some structures, the sixth ligand site is occupied by the hydroxyl group of the tyrosine residue located in the vicinity of the heme, although this site in cytochrome $P450_{cam}$ (soluble mammalian cytochrome designated as H450) is occupied by a water molecule.

Cytochromes P450 exist as numerous enzyme/isoenzyme forms having different apo-protein structures. These enzymes consist of 400—500 amino acid residues, whose

number is similar in many forms. The amino acid residue cysteine is of particular importance. This acid provides thiolate for the binding. Emphasis will be given to the redox processes catalyzed by cytochrome P450, which occupy a central place in the degradation of xenobiotics, although other reactions are also of importance. For a better understanding, it should be noted that microsomes are membrane-surrounded vesicles formed by the fragmentation of the endoplasmic reticulum (a double membrane system in the cytoplasm of eukaryotic cells). Microsomes can be isolated by differential centrifugation.

In most cases, the products that are formed in the first phase of metabolism contain functional groups (numerous examples will be given below), such as hydroxy, amino, mercapto, and carboxy groups, resulting in that xenobiotics can undergo the second phase. This is the main goal of the first phase (rather than the preparation of samples for the elimination). The goal of the second phase is to synthesize water-soluble products, which can be removed from the body by urine or bile excretion. Without going into details of chemical processes, such as various conjugations, glycosylation, sulfonation, acetylation, *etc.*, it should be emphasized that this phase results in the preparation for the excretion of drugs or other xenobiotics in some way or another.

In conclusion, let us characterize the behavior of xenobiotics, including the way of action of exogenous substances on the functional properties of the body as a whole or its parts (pharmacodynamic effects) or on the reaction of the body on the introduction of substances (pharmacokinetic effects). Therefore, the pharmacodynamics considers the biological effects of the injected drugs and the mechanisms of their biological action. The pharmacokinetics investigates the main problems associated with the absorption, distribution, deposition, metabolism, and removal of xenobiotics.

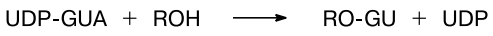
The pharmacokinetic effects have a decisive influence on the intensity and duration of pharmacodynamic effects. It should be mentioned that since the metabolism affords new compounds, which inevitably have biological activity, the effects of the substance on the body depends on which metabolites are formed in the biotransformation. Consequently, the pharmacokinetic and pharmacodynamic effects are in close relationship, and the estimation of this relationship calls for a serious analysis and experimental investigation.

Before turning to the metabolism of different xenobiotics (let us emphasize once again that most attention will be given to drugs belonging to azaheterocycles), let us consider some general problems associated with the enzyme catalysis of their degradation in the body and the relationship of the pharmacodynamic characteristics of the action of exogenous substances on the body, on the one hand, and the pathways and results of their biotransformation, on the other hand.

Most of drugs are metabolized by the body immediately after the injection. There are two aspects of this metabolism. The first aspect is the rate of metabolism. If the drug is metabolized too fast, it can be ineffective. Another important aspect is the nature of metabolites, which are formed after the introduction. In the case of high toxicity of metabolites, the drug design should be stopped. The standard method for determining the metabolic pathway is based on the injection of a radioactively labeled new drug and the subsequent analysis of labeled substances in urine and faeces[5] (other general aspects were described in the literature[6]).

Genetically mediated individual differences in the effects of drugs are reasonable because all pharmacodynamic and pharmacokinetic processes and the metabolism of drugs are mediated by protein structures, receptors, ion channels, carriers, secondary messenger systems, enzymes of the synthesis and metabolism of endogenous receptor ligands, various modulators (including peptide modulators), and drug-metabolizing enzymes. These mechanisms are DNA-dependent, which determines their individuality. In the real life, the response to drugs is determined by genetic and external factors, but the acquired properties modify the genetically dependent mechanisms. The conjugation with glucuronic acid imparts higher polarity and better water solubility to the conjugated compounds, thus enhancing their excretion (Scheme 3).

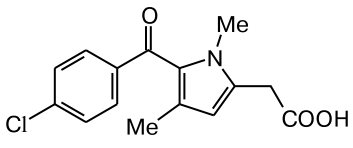
Scheme 3



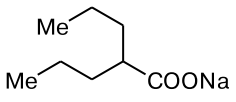
UDP is uridine phosphate, GUA is glucuronic acid, GU is glucuronide

In some cases, glucuronides can be involved in subsequent metabolic processes and can cause toxic, including cancerogenic, effects.

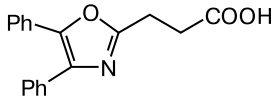
The toxic immune response was described for some drugs derived from carboxylic acids, such as the sedative agent Zomepirac, the antilipidemic agent Clofibrate, the anti-epileptic agent Sodium Valproate, and the anti-inflammatory drug Oxaprozin.



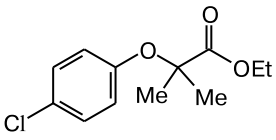
Zomepirac



Sodium Valproate



Oxaprozin



Clofibrate

The differences in the individual effects of antidepressants have been found already in the early steps of their use, when groups of patients sensitive to either tricyclic antidepressants or monoamine oxidase (MAO) inhibitors were revealed. The monoaminergic system providing either the degradation or preservation of amines is the main target for the presently known antidepressants. Tricyclic antidepressants block the reuptake of serotonin and/or noradrenaline. The MAO inhibitors have an effect on the metabolism of serotonin, noradrenaline, and dopamine. Latest-generation antidepressants have a selective effect on the transport protein mediating the transport of serotonin from the synaptic cleft back to cells. These are selective serotonin reuptake inhibitors (SSRI). The primary mechanism of action of these compounds involves the enhancement of serotonin neurotransmission. Hence, the genes controlling the serotonin pathways are of particular importance for the elucidation of the factors responsible for the individual response to antidepressants and the prediction of their effects. Selective serotonin reuptake inhibitors, which were most well studied in terms of pharmacogenetics, are used for the treatment of depressions and some anxiety and panic attacks. All these inhibitors can block serotonin receptors with different degrees of efficacy and selectivity.

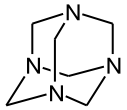
Benzodiazepine tranquilizers are main agents used in the pharmacotherapy of anxiety disorder. The mechanism of their action is based on the enhancement of the transmission by γ-aminobutyric acid (GABA), which is the key inhibitory neurotransmitter in the mammalian central nervous system (CNS). In different areas of the brain, from 20 to 50% of nervous synapses contain GABA. Three types of GABA receptors were identified; however, the GABA-A receptor containing the benzodiazepine-binding site as a component is most widespread in the brain. The GABA-A receptor is the protein complex consisting of five subunits that form the central pore passing across the cell membrane. The GABA binding to receptors leads to conformational changes of the complex, resulting in the opening of the pore, thus allowing the penetration of chloride ions into cells, which causes depolarization and hinders the excitation of cell membranes. Barbiturates, anesthetics, alcohols, and neurosteroids can bind to the receptor complex, which causes the opening of the chlorine channel. Unlike these substances, benzodiazepines act as allosteric modulators of the GABA-recognition sites and, consequently, can enhance or weaken the inhibitory action of neuromediators without the direct action on the chlorine channel.

In the simplest case (not always often), where the xenobiotic metabolism occurs through one pathway to give the only metabolite, the following situations are possible: 1) neither xenobiotic nor its metabolite exhibit significant biological effects; 2) only the xenobiotic is biologically active; 3) both a substance introduced into the body and its metabolite are active; 4) only the metabolite is active.

The latter case is of particular interest because it deals with the so-called prodrugs.

The drug metabolism is mainly the way by which the body breaks down a drug. However, in often cases the metabolic transformations afford substances with greater biological activity. For different reasons, metabolites cannot often be used directly. For example, metabolites cannot be effectively delivered to the target (penetrate through the corresponding membranes) or are insufficiently stable to act as individual drugs.

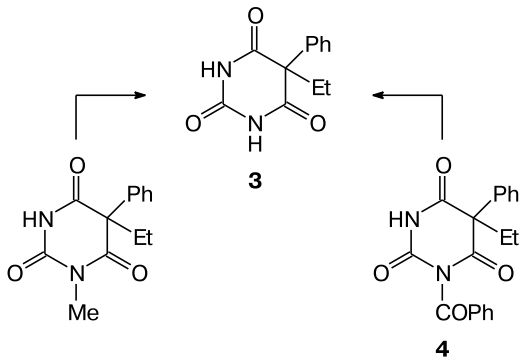
The known urinary antiseptic Urotropinum is an example of prodrugs. In essence, Urotropinum serves to deliver formaldehyde that is formed from Urotropinum under the action of acids in the urine.



Urotropin

1-Methylphenobarbital is easily transformed into phenobarbital (**3**) in the liver endoplasmic reticulum (Scheme 4). The same process is typical also of other *N*-methyl-substituted derivatives of heterocycles.

Scheme 4



Phenobarbital (**3**) is still (although it was used for the first time at the beginning of the 20th century) one of the most effective antiepileptic drugs, which specifically suppreses high-voltage high-frequency discharges in the brain experienced during an epileptic seizure.

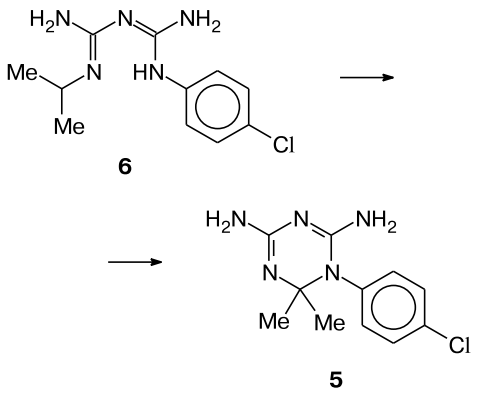
Benzonal (**4**) can easily be transformed into phenobarbital (**3**) by 1-debenzoylation, which readily occurs in aqueous media.

Actually, the investigation of the pharamacokinetics and metabolism of benzonal (**4**) showed that this drug is rapidly metabolized in the body to give phenobarbital (**3**), which exerts anticonvulsant effects. Therefore, benzonal (**4**) is the prodrug.

The true drug, *viz.*, dihydrotriazine derivative **5**, is produced in the body under the treatment with the antimalarial drug Bigumal (**6**) (Scheme 5).

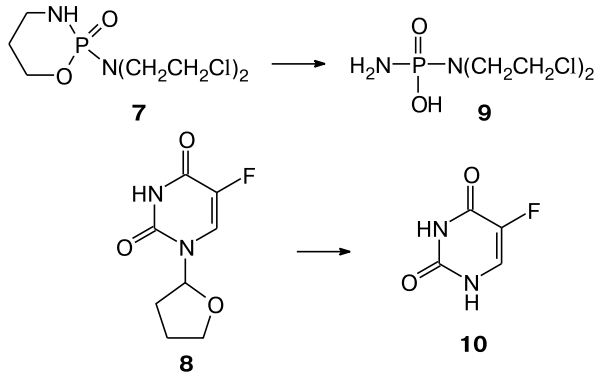
Analogous processes associated with the formation of more active compounds are observed under the treatment with antitumor drugs, such as Cyclophosphane (**7**) and

Scheme 5



Fluorofur (**8**). As a result, phosphoramide **9** and the known drug 5-Fluorouracil (**10**) (Scheme 6) are produced.

Scheme 6



Metabolic transformations of heterocycles

It is reasonable to separately consider heterocyclic compounds primarily because heterocycles dominate among various drugs.

It should be noted that a knowledge of the chemical structures of true drugs is of considerable importance because it provides a route to the synthesis of structures most suitable (most complementary) for interactions with a particular receptor system.

It is very important that enzyme systems specific for endogenous substrates are actively involved in the degradation of xenobiotics.

Cytochrome P450-mediated reactions are most common in the phase I metabolism. These oxidative processes have a central place in the degradation of xenobiotics.

When discussing the drug metabolism, it should bear in mind that in many cases various parallel and successive reactions occur to give numerous products whose properties often differ from those of the initial molecule. As an illustration, the catabolism of the new antipsychotic agent Mazapertine (**11**)[7] is presented in Schemes 7 and 8. This catabolism involves a series of processes, such as hydroxylation, dealkylation, deacylation, amidation, dearylation, debenzylation, *etc*. It should be noted that not all these processes can easily be performed by non-enzymatic pathways. For example, it is impossible to introduce hydroxy groups into the piperidine ring without using an enzyme. It should be noted that the selective dealkylation in the case of such a large number of functional substituents without the involvement of enzymes is also a non-trivial problem for synthetic chemists.

Hereinafter, the metabolism of heterocycles will be considered in relation to the ring size of the substrates under study.

Small rings. Let us start with aziridine derivatives. Yet another monooxygenase system involved in the metabolism of xenobiotics, *viz.*, microsomal flavin-containing monooxygenases (FMO), deserves attention. The properties of these enzymes are similar to those of flavoprotein monooxygenases found in bacteria, *viz.*, *p*-hydroxybenzoate hydroxylases, salicylate hydroxylases, cyclohexanone oxygenases, and luciferases. The catalytic cycle of FMO begins with the binding of NADPH and the reduction of flavin to $FADH_2$.[8] In addition, FMO mediates also the oxidation of aziridines to unstable *N*-oxides, which are decomposed to form olefins and nitrosoalkanes (Scheme 9).[4]

Azetidine derivatives also form *N*-oxides (Scheme 10).

The major *in vivo* metabolite of Tazadolene (**12**), which is a non-opioid analgesic with antidepressant properties, is formed through the cleavage of the strained azetidine ring (Scheme 11).

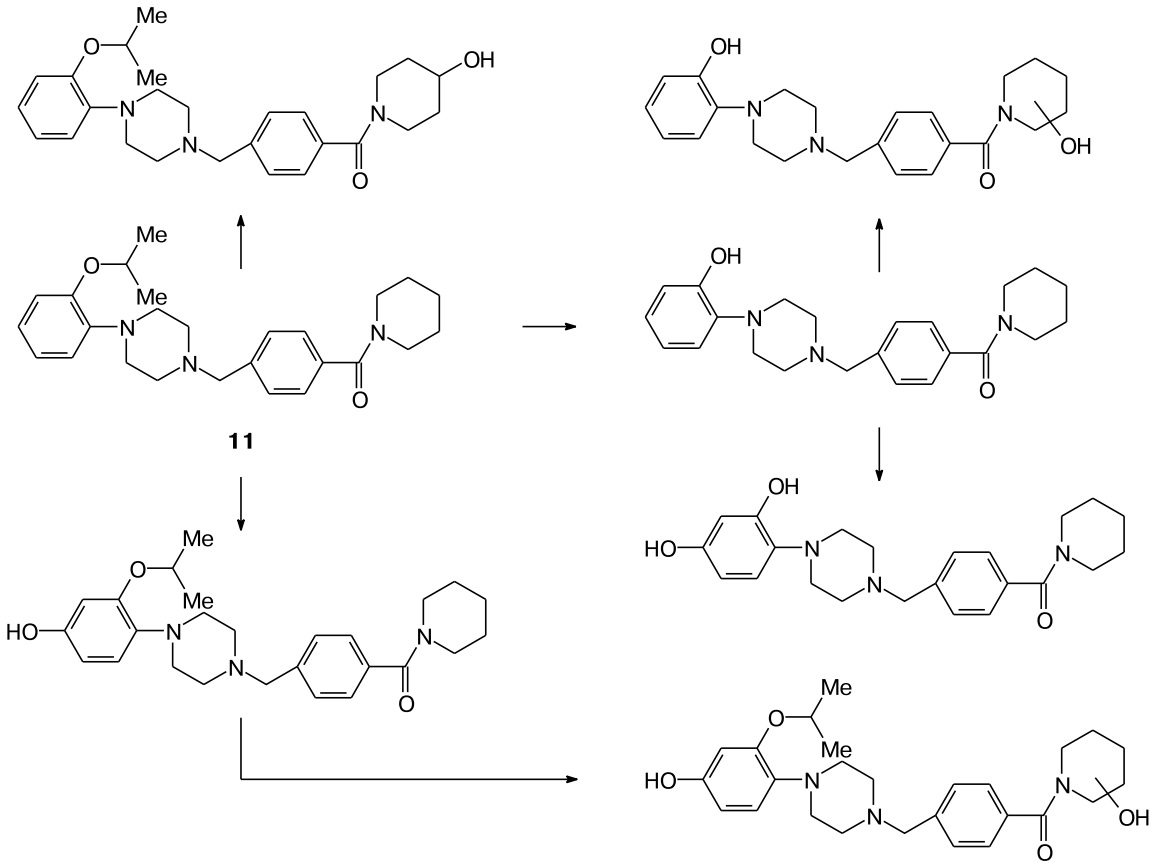
Five-membered heterocycles. The study of the metabolism of 3-(*p*-chlorophenyl)pyrrolidine (**13**), which is the prodrug of the myorelaxant Baclofen, showed that its transformation follows two pathways (Scheme 12)[9] to give Baclofen (**14**) and Baclofen Lactam (**15**) (path *A*) or α-Baclofen (**16**) and α-Baclofen Lactam (**17**) (path *B*). The path *A* is preferred over the cytochrome P450-catalyzed oxidation (path *B*) due apparently to steric factors.

The transformations of xanthine derivatives, such as the drugs Caffeine (**18**), Theophylline (**19**), and Theobromine Sodium Salt (**20**), are catalyzed by the enzyme xanthine oxidase (Scheme 13)[10] and afford uric acid (**21**).

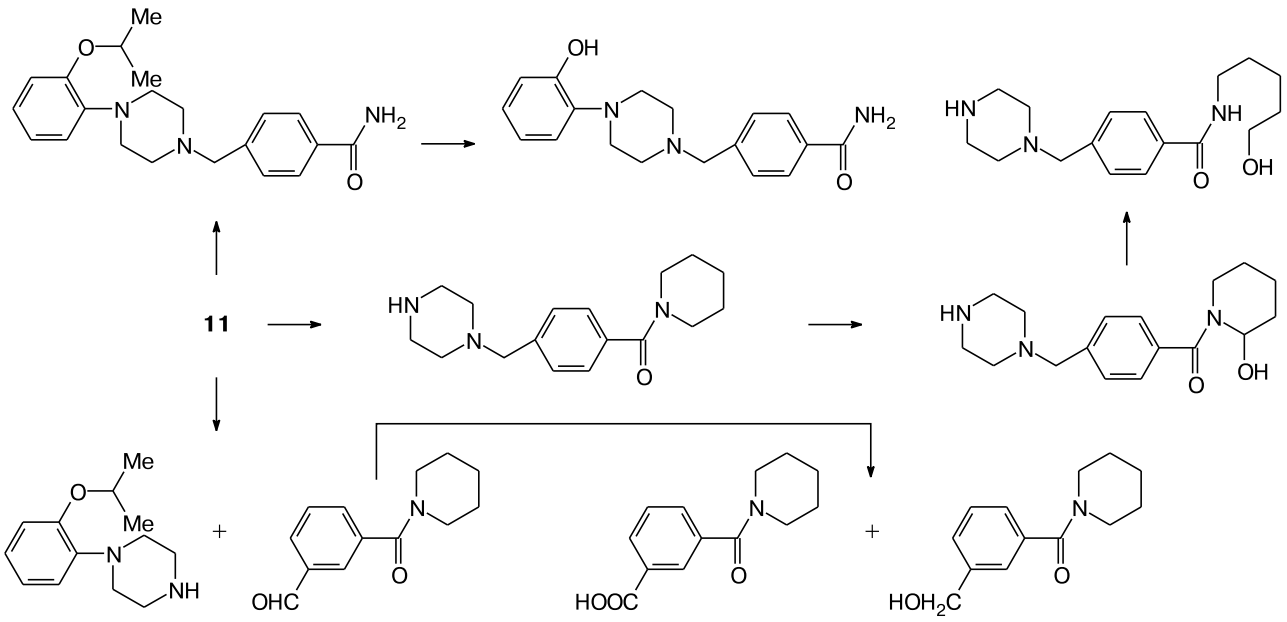
Saturated (or partially hydrogenated) heterocycles are subjected to cytochrome P450-catalyzed oxidation resulting in the cleavage of N—C bonds. Thus, 2-(α-pyridyl)-2-phenylethylimidazoline (**22**) injected into rats is oxidized to a number of compounds that are formed through the cleavage of the imidazoline ring (Scheme 14).

The metabolism of furan derivatives typical of many five-membered heterocycles, including nitrogen-containing heterocycles, was considered in the study.[2] Two pronounced metabolic pathways are observed for furan deriv-

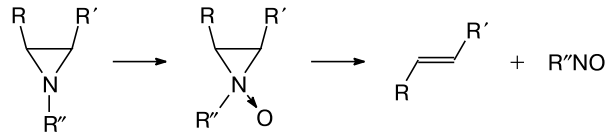
Scheme 7



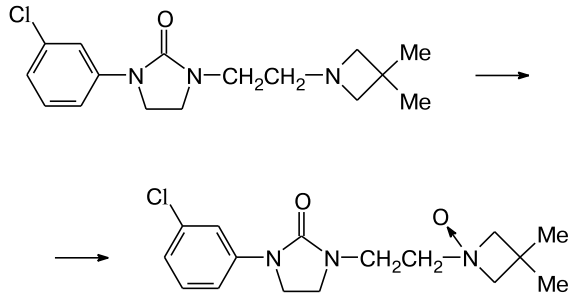
Scheme 8



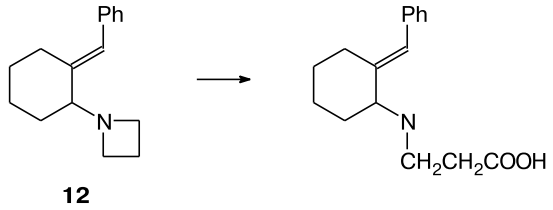
Scheme 9



Scheme 10

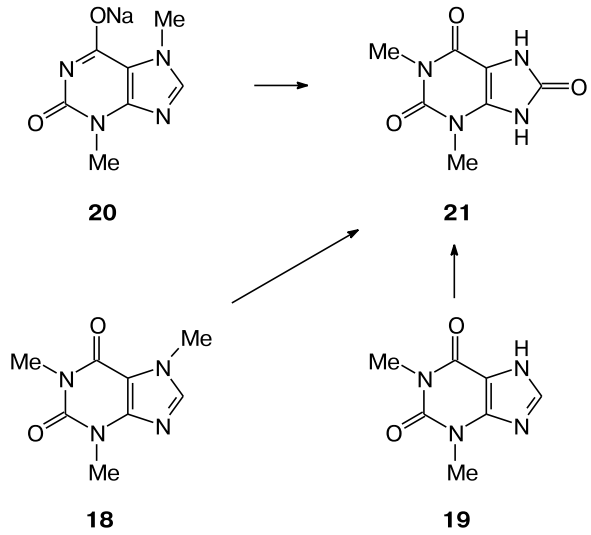


Scheme 11



12

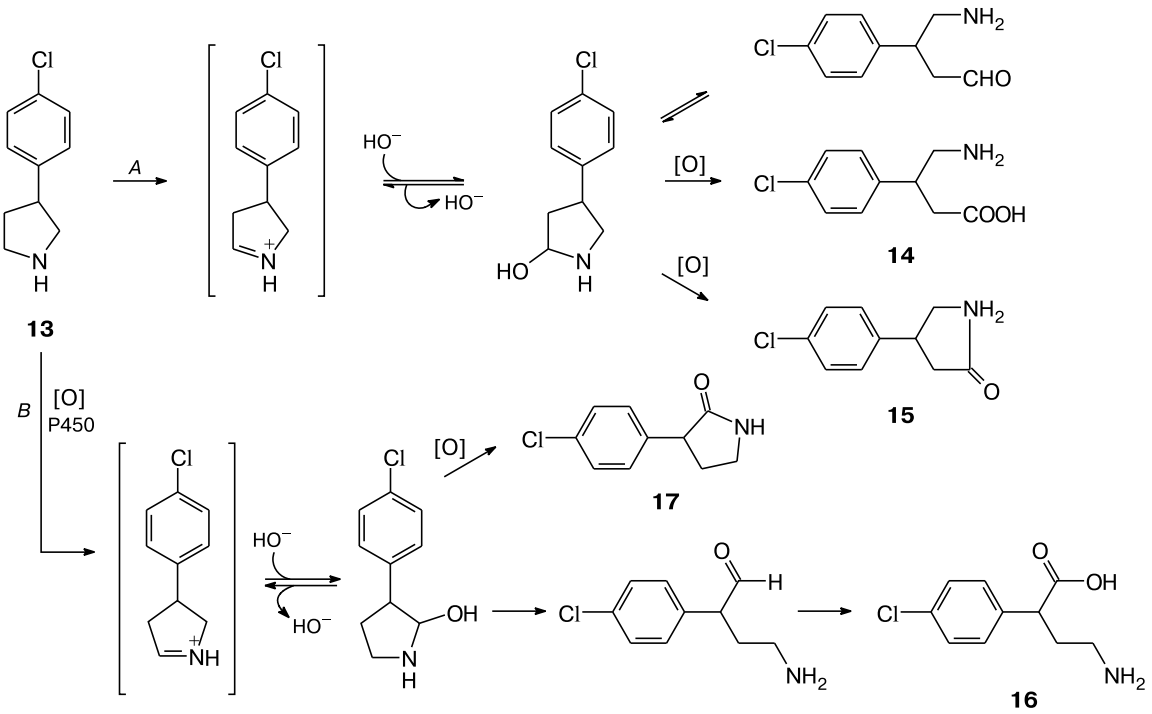
Scheme 13



20 **21** **18** **19**

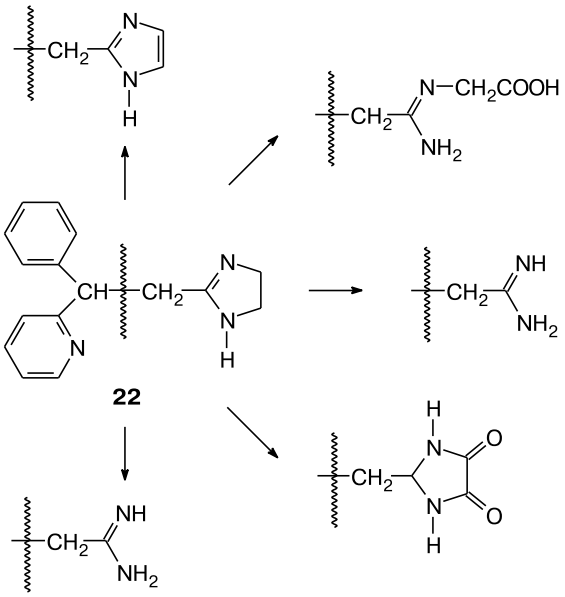
atives **23**. One of these pathways (*a*) is based on the 4,5-epoxidation giving rise to 5-hydroxy derivative **24** followed by the rearrangement into lactone **25**, whose hydrolytic cleavage affords the corresponding γ-keto acid **26**. Another pathway (*b*) involves the oxidative ring opening to give an unsaturated keto aldehyde. Metabolites of this type are highly active electrophilic reagents capable of covalent binding to tissue macromolecules (Scheme 15).[2]

Scheme 12

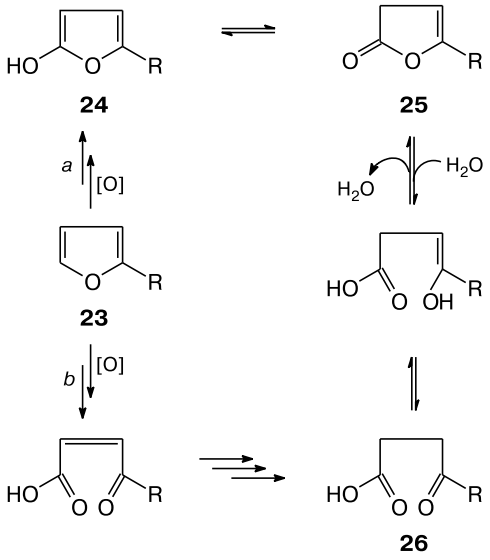


13 **14** **15** **17** **16**

Scheme 14



Scheme 15

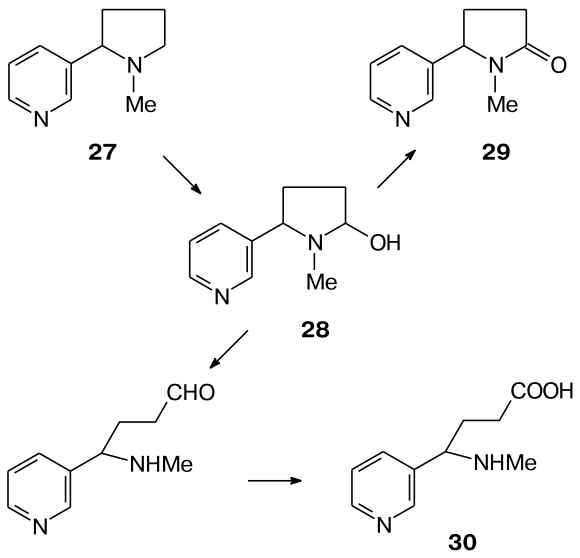


Scheme 16 reflects the metabolic pathways associated with the biodegradation of the alkaloid nicotine (**27**)[11] involving the formation of the corresponding derivatives of carbinolamine (**28**), lactam (cotinine, **29**), and amino acid (**30**).

As is evident from the structure of the substrate (nicotine), the oxidation can afford (Scheme 17) either 1′,2′-(**31**) or 1′,5′-dehydro cations (**32**), which undergo further transformations to form products of different structures.[2,11]

Let us emphasize once again that the key step of the process under consideration affords carbinolamines, which

Scheme 16



are often unstable. These compounds undergo rapid hydrolysis to give amine and formaldehyde, and the latter is oxidized by dehydrogenases to formic acid. It should be noted that some carbinolamines were detected and their conjugates, for example, glucuronides, were identified. Thus, *N*-methylcarbazole (**33**) is spontaneously decomposed to give carbazole (**34**) and formaldehyde, but this process is strongly accelerated by different animal species liver microsomes (Scheme 18).[1]

Analogous schemes were suggested also[2] for other pyrrolidine derivatives, such as Tremorine (**35**) and Prolintane (**36**) (Scheme 19).

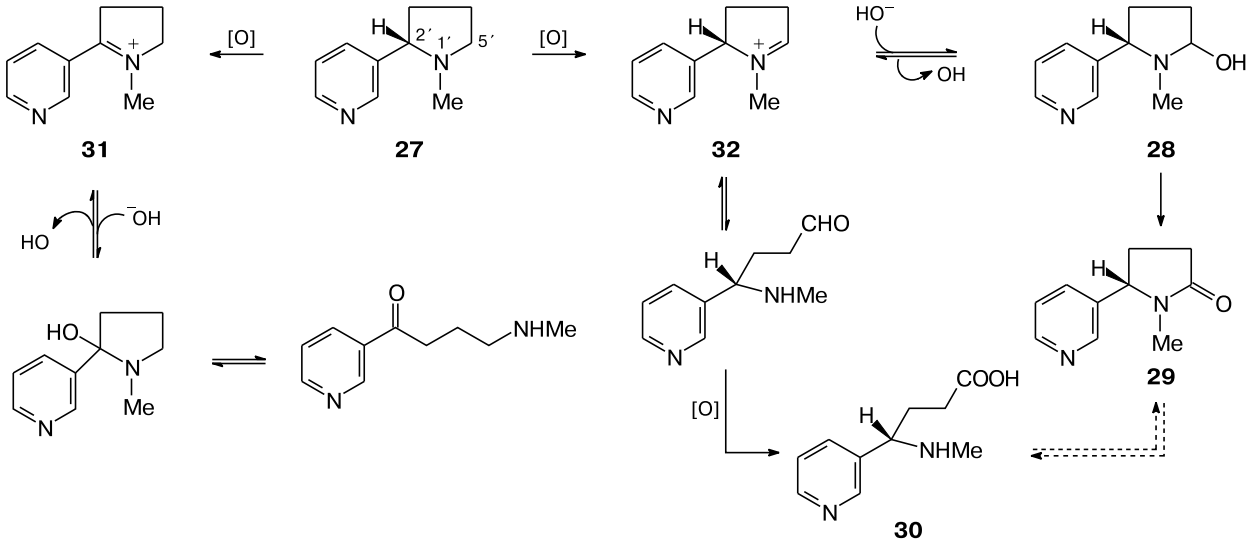
The pyrimidine derivative, *viz.*, 4,6-bis(pyrrolidino)-pyrimidine (**37**), is transformed into relatively stable carbinolamine **38** upon the incubation with rat liver microsomes (Scheme 20).[12]

One of the metabolites of 3-methylindole (**39**) is 3-hydroxy-3-methyloxindole (**40**). The route to this bicyclic lactam involves the initial P450-mediated formation of epoxide followed by the oxirane ring cleavage to form the iminium ion **A** (Scheme 21),[2] which undergoes the transformation into methyloxindole **40** catalyzed by alcohol oxidase (AO).

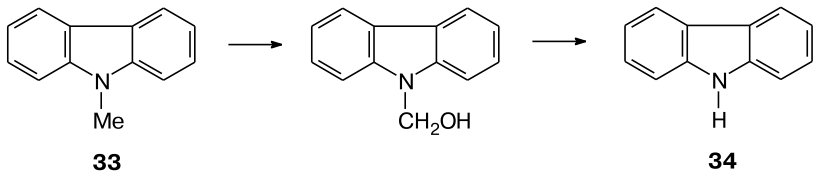
The antiepileptic drug Zonisamide **41** is an example of compounds containing the N—O bond in the heterocycle. The reductive cleavage of the latter in the body is accompanied by the cleavage of the isoxazole ring to form sulfonamides (Scheme 22).[2]

The P450-mediated oxidative transformation of indole (**42**) affords dimeric products (Scheme 23). The binding of indoxyl (**43**) on the outer side of the P450 active site gives indigo (**47**) and indirubin (**48**). It is suggested that other products, for example, such as 6*H*-oxazolo-[3,2-*a*:4,5-*b*′]diindole (**46**), are formed after the binding

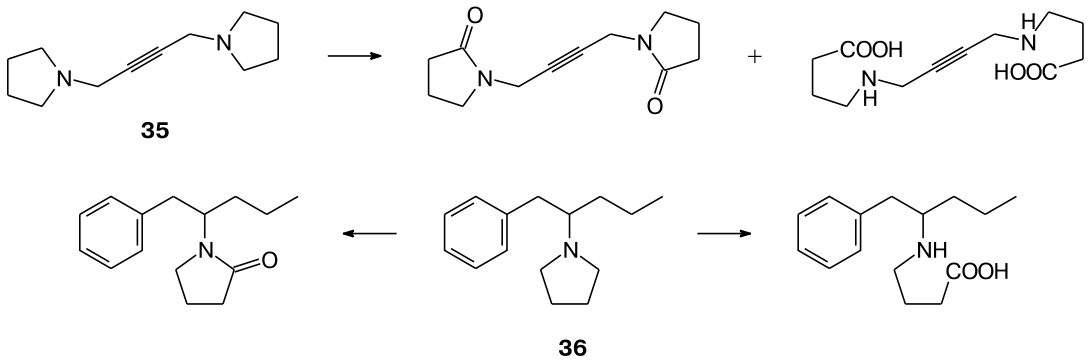
Scheme 17



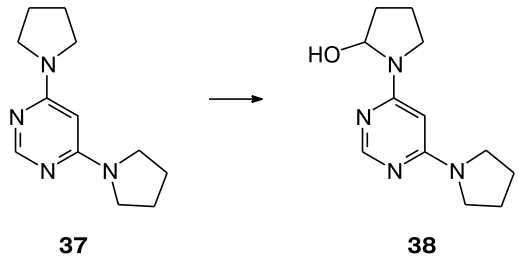
Scheme 18



Scheme 19



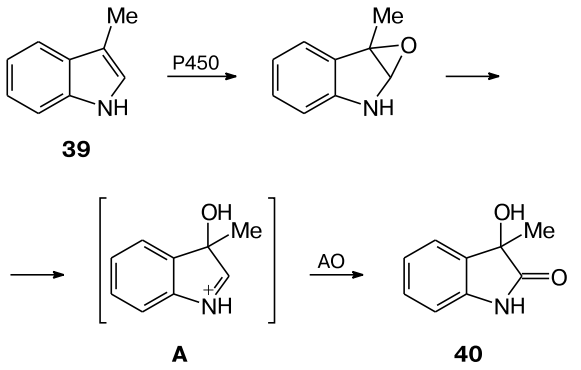
Scheme 20



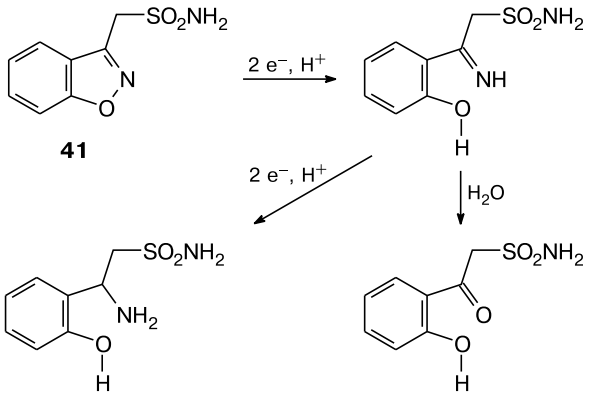
in the active site of the enzyme. It is very interesting that the above-considered oxidative processes afford such derivatives as indoxyl (**43**), oxindole (**44**), 6-hydroxyindole (**45**), isatin (**49**), and 3-hydroxyindole (**50**) (see Scheme 23).

Yet another impressive example of biotransformations giving the effective antitumor agent (antileukemic drug) 6-mercaptopurine (**51**) is the use of *S*-(6-purinyl)-L-cysteine (**52**) as a prodrug. The clinical use of 6-mercaptopurine is complicated by its fast biotransformation under

Scheme 21

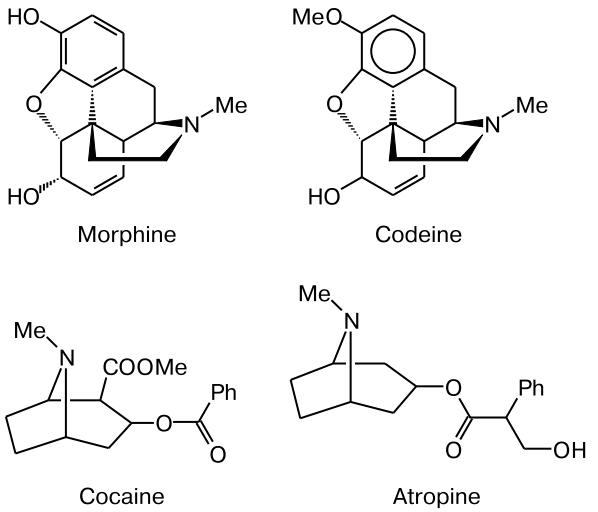


Scheme 22

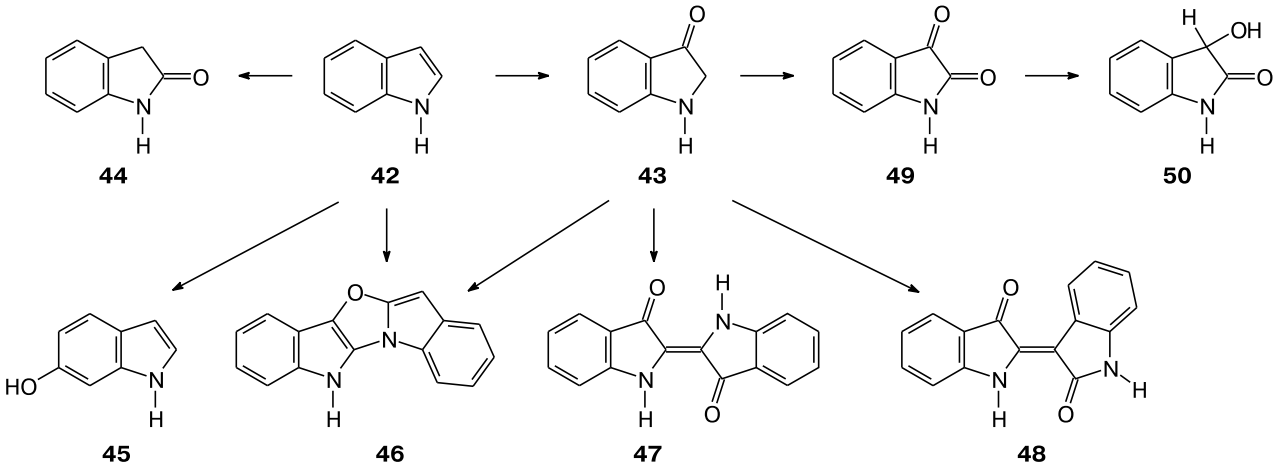


catalysis by xanthine oxidase giving inactive 6-thiouric acid (**53**). The extensive metabolism forces the use of 6-mercaptopurine in very high doses. This results in the damage of liver and other tissues and causes substantial toxic effects, which are to a large extent eliminated with the use of *S*-(6-purinyl)-L-cysteine (Scheme 24).

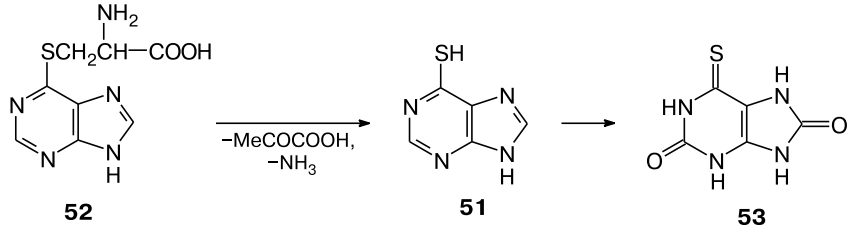
Six- and seven-membered heterocycles. Six-membered *N*-heterocycles also undergo *N*-oxidation. A large series of alkaloids, such as morphine (**54**), codeine (**55**), ethylformine, atropine, scopolamine, cocaine, and strichnine, are good substrates of FMO.

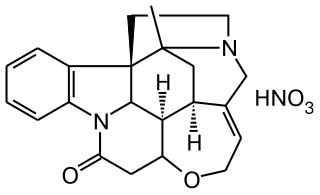


Scheme 23

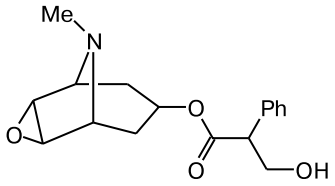


Scheme 24





Strichnine

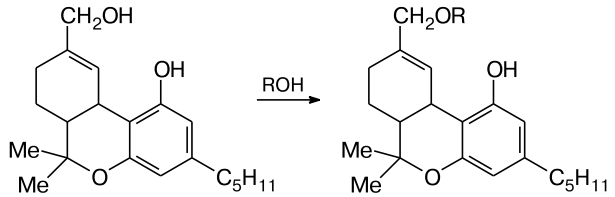


Scopolamine

By contrast, hydroxymorphine, nalorphine, naloxone, and brucine are poor substrates.[13]

The phase II metabolism involves also the conjugation with fatty acids typical of various heterocycles, including O-heterocyclic compounds. The process is exemplified by the cannabinol derivative (**56**),[2,4] which is an active ingredient of the cannabis resin (the narcotic substances marijuana and hashish from Indian hemp causing narcotic addiction). The liver microsomal fraction catalyzes the O-acylation of the 11-hydroxy group of palmitic and stearic acids (Scheme 25).

Scheme 25



56

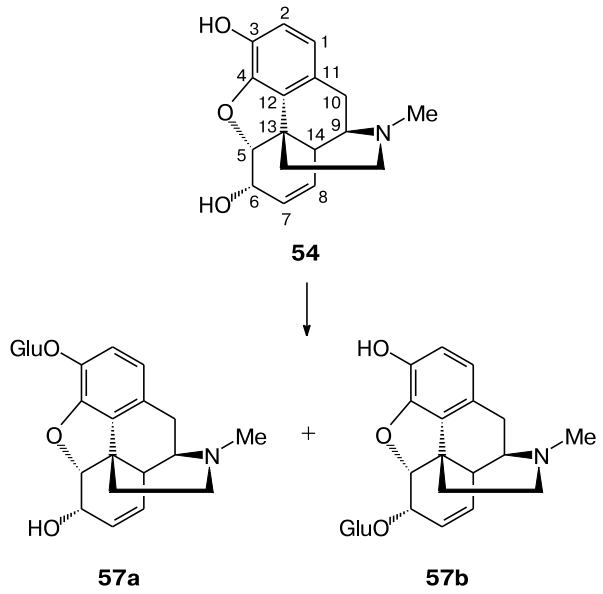
R = $CO(CH_2)_{14}Me$, $CO(CH_2)_{16}Me$

Very interesting phenomena are observed in the biotransformation of morphine (phase II metabolism).[3] The conjugation of morphine to glucuronic acid follows two pathways to give 3-β-D-glucuronide (**57a**) as the inactivation product of morphine and 6-β-D-glucuronide (**57b**), which is more active than morphine and is used as an analgesic (Scheme 26).

The biotransformation of the antipsychotic agent of the phenothiazine series, *viz.*, Sulforidazine (**58**), also affords lactam **59** (Scheme 27).[14]

Metabolites of the central nervous system stimulant Methylphenidate (**60**) contain the piperidone ring (Scheme 28).[15]

Scheme 26



57a **57b**

After injection into rats, the thrombocyte aggregation inhibitor, *viz.*, the tetrahydroimidazo[2,1-*b*]quinazolin-2-one derivative, is transformed into the metabolite of piperidone and the corresponding amino acid, with the latter predominating (Scheme 29).[15]

Other six-membered heterocycles are subjected to biotransformations similar to that characteristic of piperidines. Thus, lactam containing the piperazinone fragment is the main metabolite of Ciprofloxacin[3,4,16] (Scheme 30).

The cytochrome P450 catalysis leads to the hydroxylation of pyridine at positions 2 and 4, the transformation of pyrazinecarboxamide into the 5-hydroxy derivative (these hydroxy derivatives are stabilized in the lactam oxo form), and the hydroxylation of pyrazole at position 4 (Scheme 31).[1–4]

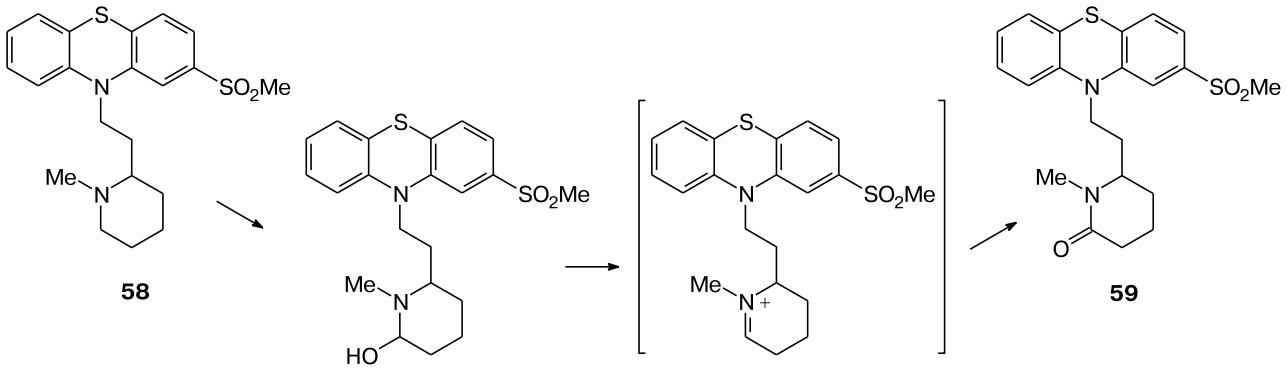
Compounds with two pyridine rings undergo the regio- and stereoselective *N*-oxidation. Thus, the diagnostic drug Metyrapone (**61**) is oxidized at both pyridine nitrogen atoms; however, the oxidation at the N atom of the ring adjacent to the carbonyl group is the major process in rat liver microsomes (Scheme 32).[17]

The enzymatic oxidation of 1,3-(4-dipyridyl)-2-phenylpropan-2-ol affords only the enantiomerically pure levorotatory isomer (Scheme 33).[18]

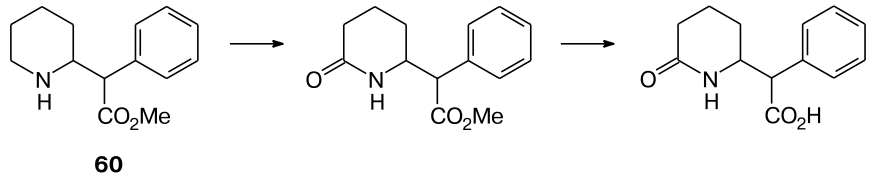
N-Oxides rather rarely exhibit the *in vitro* pharmacological activity, but they are often *in vivo* active because these compounds are metabolized in the body through the reduction of the oxide group.

Let us consider 1,4-dihydropyridines, which can be included in the group of cyclic dienamines and which belong to an important class of biologically active compounds. This class of compounds includes a large number of antihypertensive drugs, *viz.*, calcium antagonists (4-

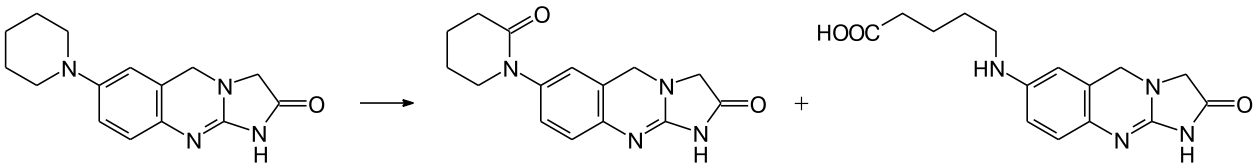
Scheme 27



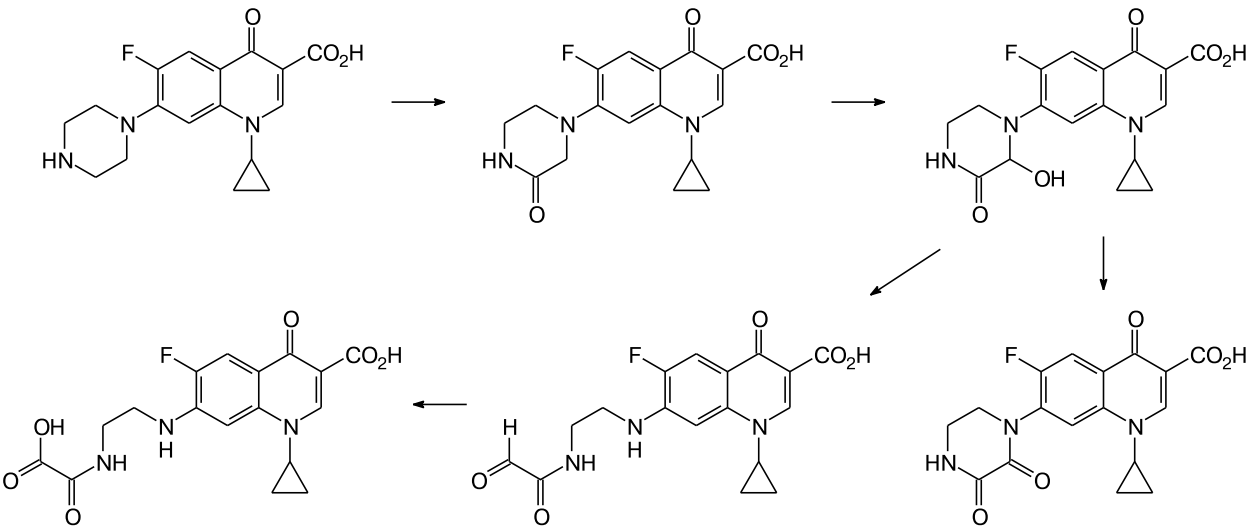
Scheme 28



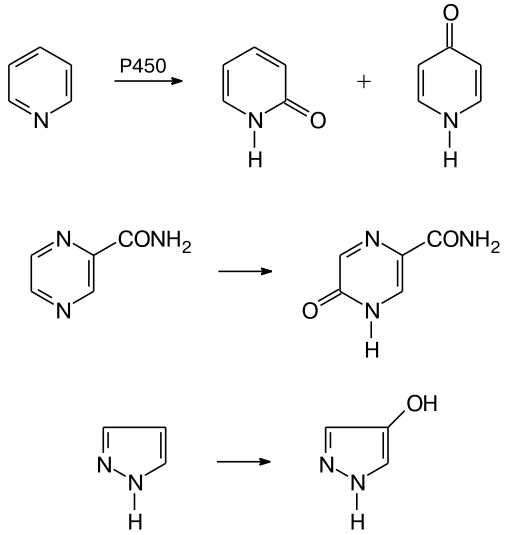
Scheme 29



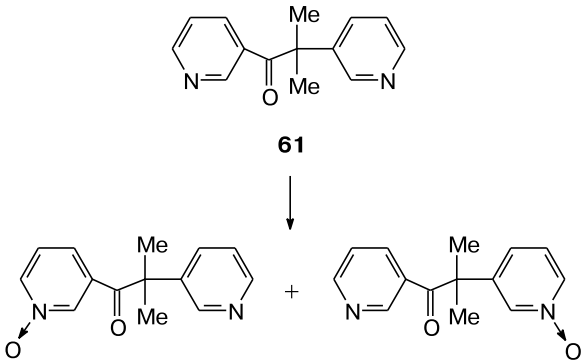
Scheme 30



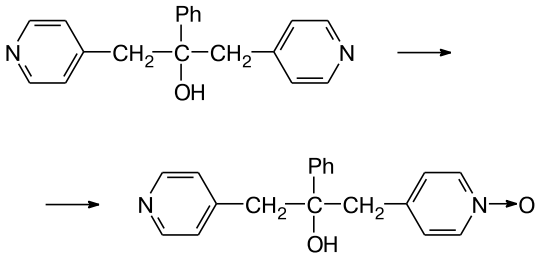
Scheme 31



Scheme 32



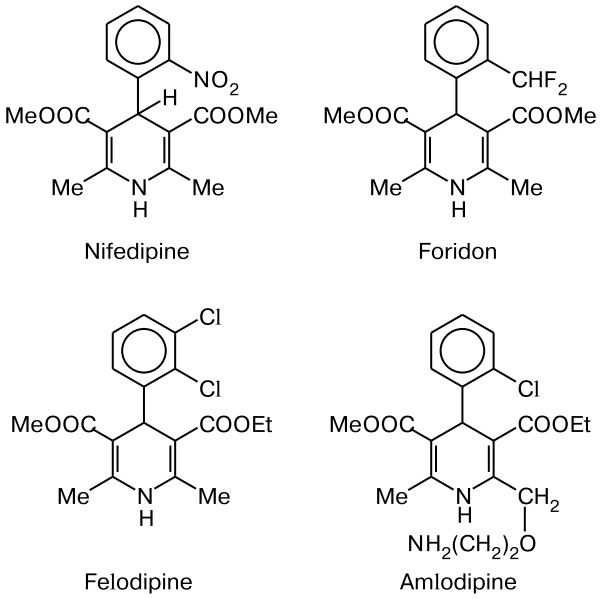
Scheme 33



aryl derivatives, such as Nifedipine, Foridon, Felodipine, and Amlodipine).

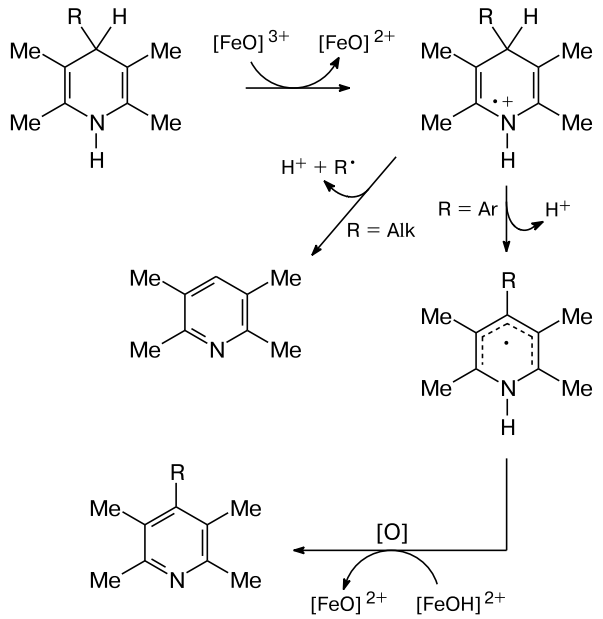
Dihydropyridines containing alkyl substituents at position 4 of the pyridine ring exhibit properties of irreversible cytochrome P450 isoenzyme inhibitors.

Dihydropyridine derivatives serve as substrates of cytochrome P450, which are subjected to aromatization

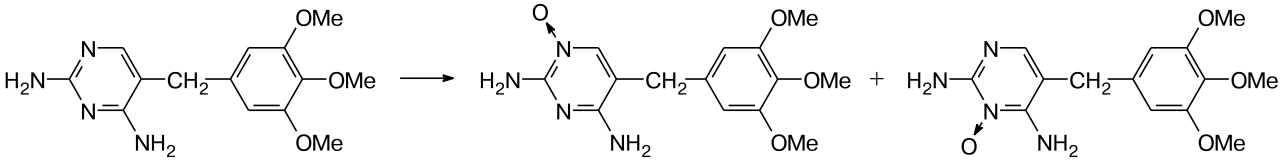


in one of the stages of metabolism (after the oxidative stage).[19,20] The initial stage involves the one-electron oxidation of the nitrogen atom to form the aminium radical cation. The further transformations of this radical cation depend on the nature of the substituent at position 4. In the presence of the alkyl group, the radical cation is subjected to aromatization to give the alkyl radical; in the presence of the aryl group, the proton abstraction is followed by the cytochrome P450-mediated oxidation to give finally the 4-arylpyridine derivative (Scheme 34).[21]

Scheme 34



Scheme 35

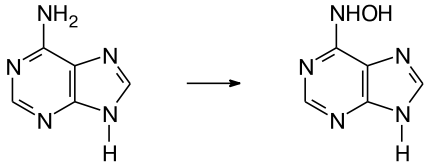


62

The metabolism of hetarylamines can be exemplified by the oxygenation of the antibacterial drug Trimethoprim (**62**). It was found that the hydroxylation, which is generally observed for benzene derivatives, does not occur in the latter case; instead, two N-oxides at the N(1) and N(3) atoms are formed (Scheme 35).[22]

However, the metabolism of adenine (**63**) occurs through another pathway to give the hydroxylamine derivative, *viz.*, *N*-(6)-hydroxyaminopurine (**64**) (Scheme 36).[1,2,4]

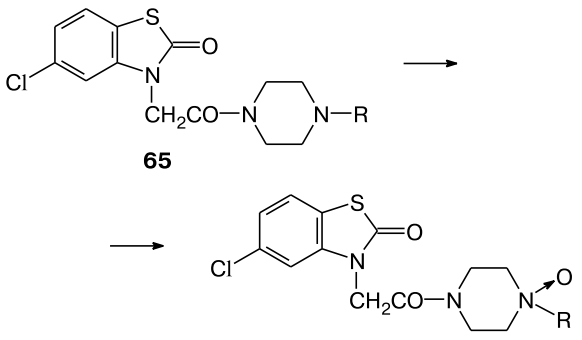
Scheme 36



63 **64**

Tiaramide (**65**) is a new anti-inflammatory drug, which is also metabolized to N-oxide (Scheme 37).[2]

Scheme 37

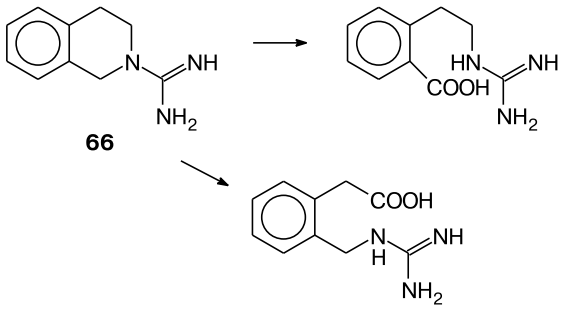


65

The ring opening (in the case under consideration, the opening of the saturated six-membered ring) is typical of some biodegradation pathways of the antihypertensive drug Debrisoquine (**66**) (Scheme 38).[23–25]

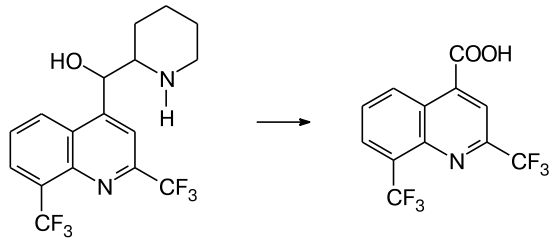
The oxidative degradation of the antimalarial drug Mefloquine (**67**) affords 2,8-bis(trifluoromethyl)quinoline-4-carboxylic acid (Scheme 39).[26a]

Scheme 38



66

Scheme 39



67

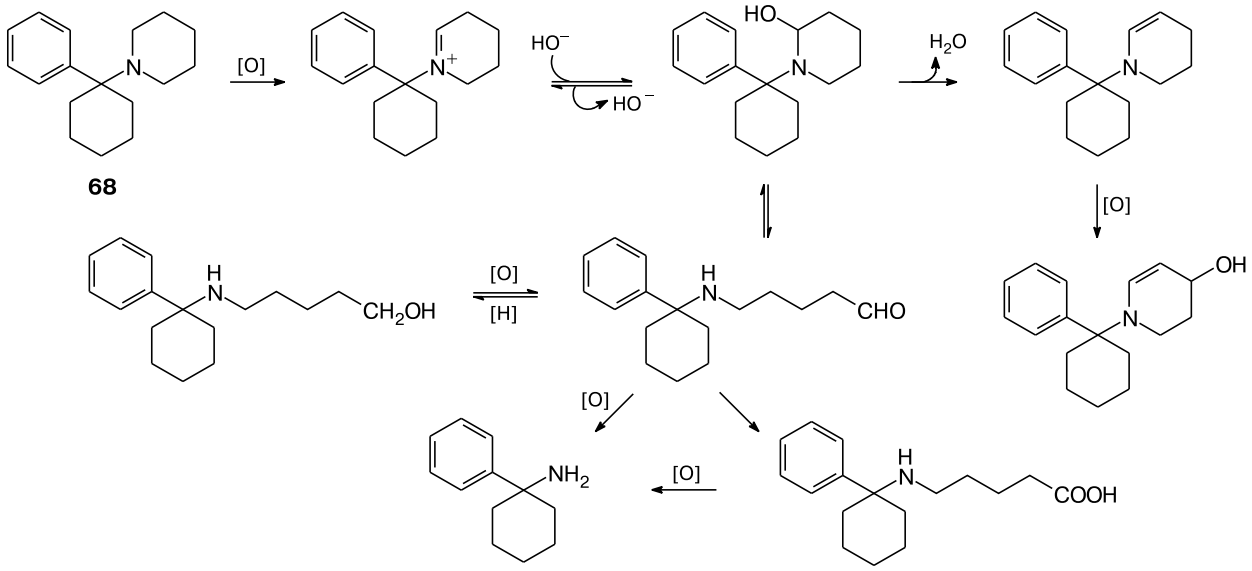
The oxidative metabolism of the anesthetic and analgesic drug Phencyclidine (**68**) giving various metabolites and intermediates is shown in Scheme 40.

It should be noted that some compounds produced by the biotransformation have a strong toxic effect.[26b]

Amine oxidases[26c] are divided into several classes: monoamine oxidases (MAO) responsible for the metabolism of catecholamines, diamine oxidases (DAO) catalyzing the metabolism of endogenous diamines, for example, of histamine, as well as flavoprotein *N*-oxidases and flavoprotein hydrolases.

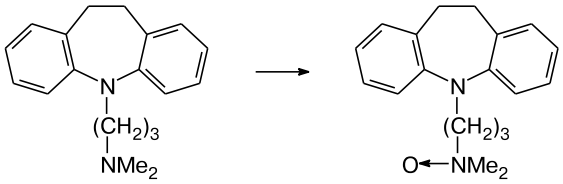
Monoamine and diamine oxidases are involved predominantly in the metabolism of endogenous substances; N-oxidases are important for the drug metabolism. An example is the antidepressant Imipramine (**69**), which is the non-selective reuptake inhibitor of the neuromediators noradrenaline, dopamine, and serotonin. N-Oxidases are located in liver microsomes. These enzymes exhibit catalytic activity in the presence of NADPH and molecular oxygen. The oxidation of Imipramine (**69**) occurs at the

Scheme 40



exocyclic dimethylamino group to form the corresponding aliphatic N-oxide[27] (Scheme 41).

Scheme 41



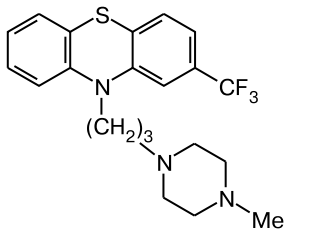
69

Many drugs, for instance, the psychotropic agents, such as the neuroleptic agent Trifluoperazine, the antipsychotic medication Fluphenazine, the antiemetic agent Prochlorperazine, and the tranquilizer Perphenazine, contain the piperazine fragment.

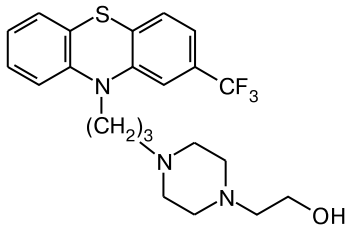
The chronic injection of these drugs into rats and dogs leads to the accumulation of metabolites containing the ethylenediamine fragment in tissues. The general scheme of the degradation of these drugs in the body is shown in Scheme 42.[2]

The analgesic and anti-inflammatory drug Emorfazone (**70**) contains the morpholine moiety whose oxidation is the major process in the metabolism of this drug (Scheme 43).[28]

In this context, it is reasonable to consider the known anticancer drug Cyclophosphane (**7**) once again.[29] As mentioned above, Cyclophosphane is a prodrug whose degradation ensures the activation and the cytotoxic effect predominantly in hypoxic tumor cells and the deactivation in healthy cells.

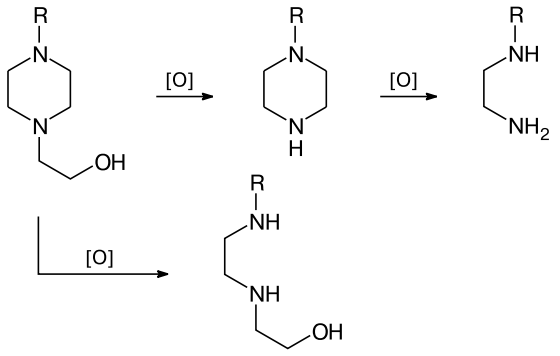


Trifluoperazine

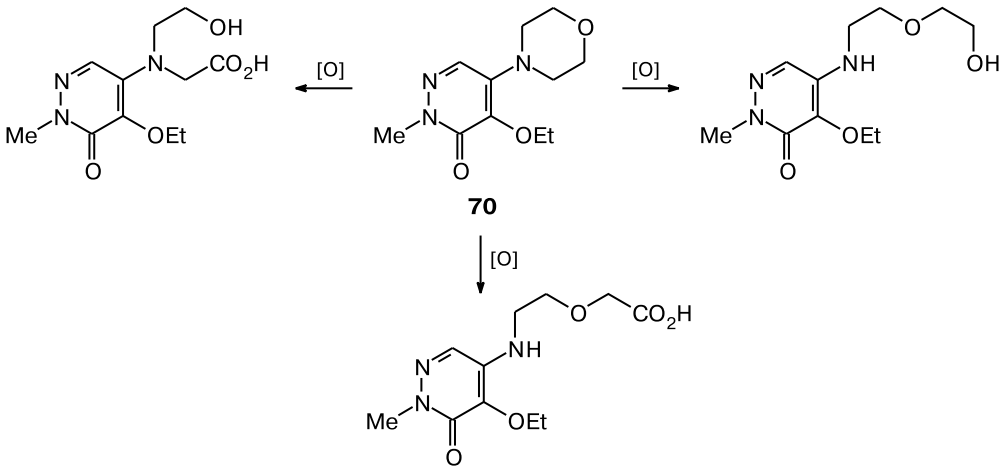


Fluphenazine

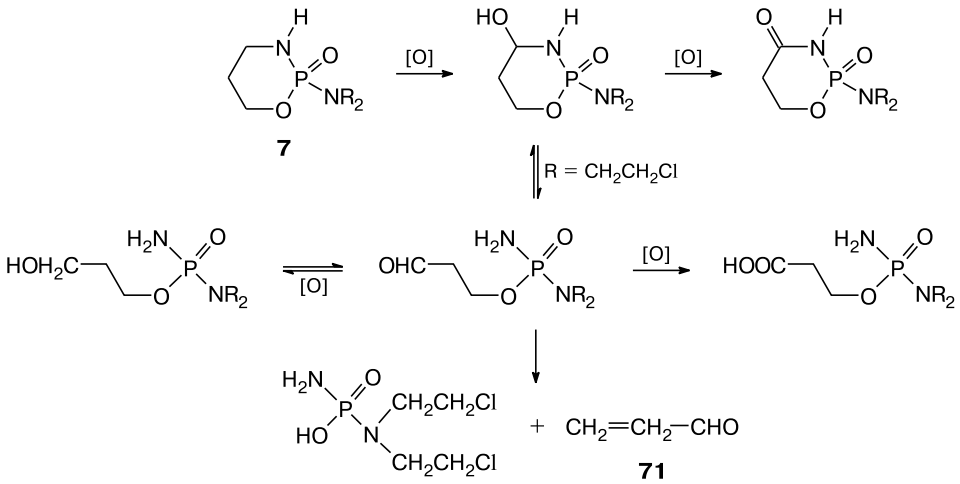
Scheme 42



Scheme 43



Scheme 44



The biodegradation of Cyclophosphane (**7**) occurs *via* the P450-catalyzed oxidation to give a number of acyclic phosphoramide compounds. The metabolism affords also acrolein **71** (Scheme 44).[29]

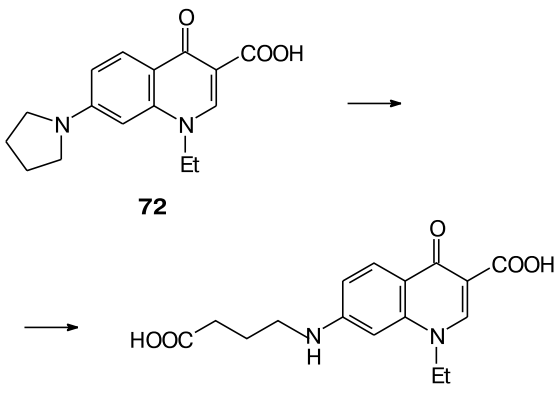
The metabolism of the antibacterial drug Piromidic acid (**72**) does not produce lactam due to steric hindrance and only an amino acid derivative is formed by the metabolism[2] (Scheme 45).

The antihypertensive drug Debrisoquine (**66**) undergoes the oxidation at the benzylic methylene unit at position 4 both in human and animals (Scheme 46).[23–25]

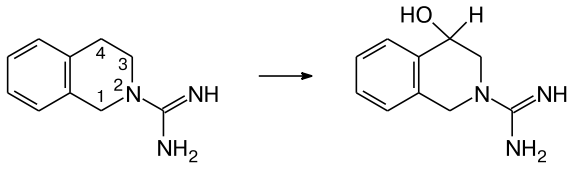
The methylene groups at positions 1 and 3 are also oxidized to form hydroxy and then oxo derivatives. The further cleavage of the lactam rings affords the corresponding acids (Scheme 47).

The sedative and hypnotic drug Zaleplon (**73**) is metabolized through the oxidation at the pyrimidine ring to form heterocyclic lactam containing the pyrimidine moiety (Scheme 48).[2]

Scheme 45

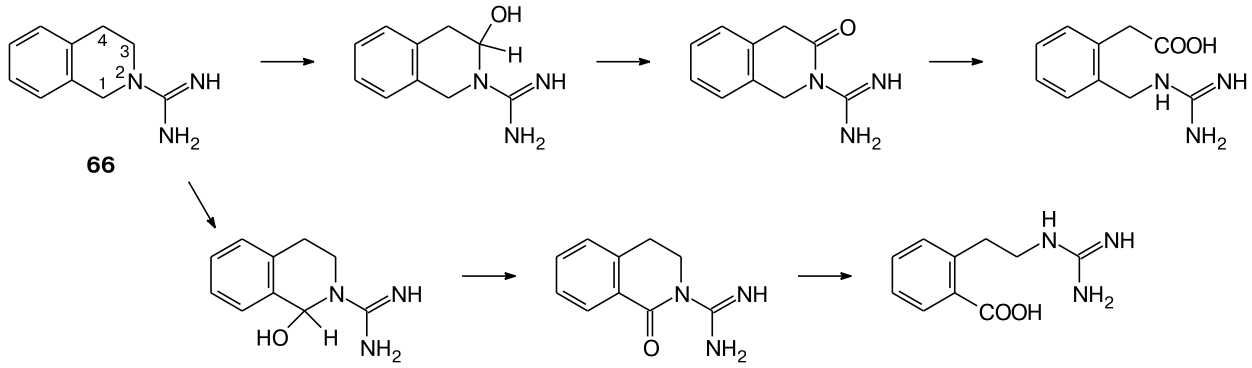


Scheme 46



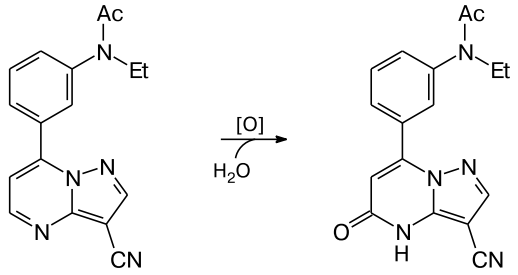
66

Scheme 47



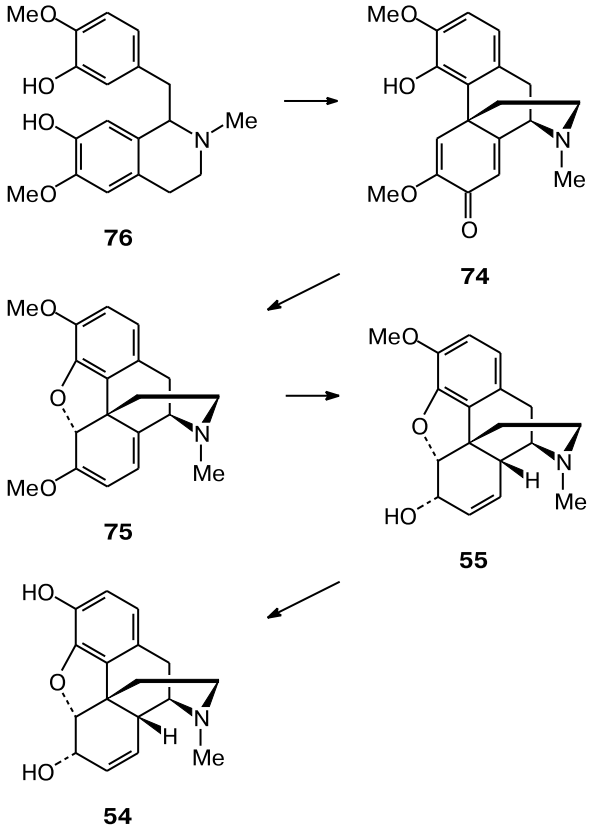
66

Scheme 48



73

Scheme 49



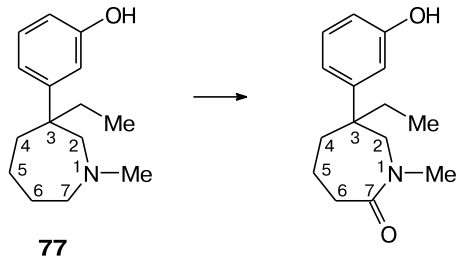
76 **74** **75** **55** **54**

The formation of the bond between the six-membered cyclic systems is observed in the oxidation of the alkaloid salutaridine (**74**). Scheme 49 shows the pathway of subsequent transformations of this alkaloid through thebaine (**75**) and codeine (**55**) into morphine (**54**). The formation of salutaridine (in liver and plant microsomes) assumes the involvement of reticuline (**76**).

The enzymatic oxidation of the narcotic analgesic drug Meptazinol (**77**) occurs regioselectively to form only the 7-oxo derivative (Scheme 50). The 2-oxo metabolite was not found and its absence is apparently attributed to steric factors.[2]

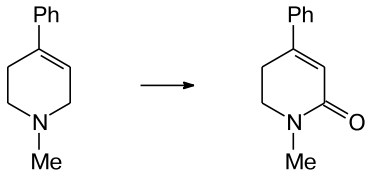
For the known neurotoxin 1-methyl-4-phenyltetrahydropyridine (MPTP) that causes Parkinson´s disease in human and mice, the detoxification by monoamine oxidase B affords the corresponding lactam (Scheme 51).[30]

Scheme 50



77

Scheme 51

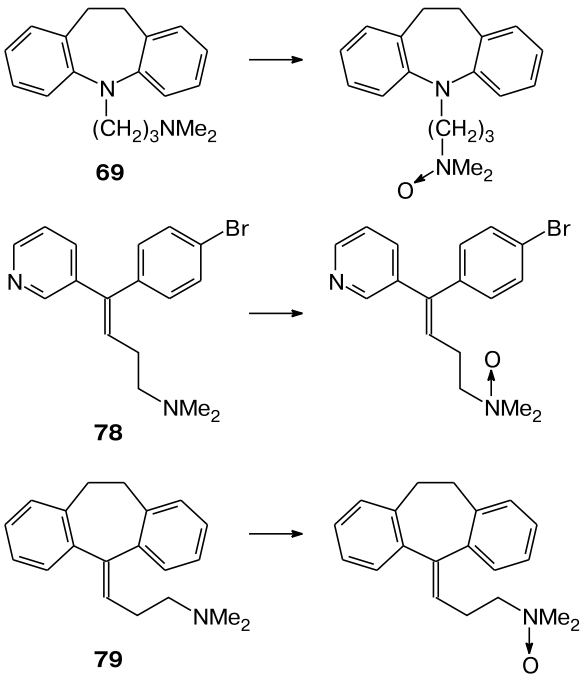


MPTP

Some other important metabolic processes

The N-oxidation, N-alkylation, N-dealkylation, and other processes[31—36] are not only associated with heterocyclic compounds and drugs but are also related to other types of compounds, *i.e.*, are in many aspects common to the metabolism of organic compounds.

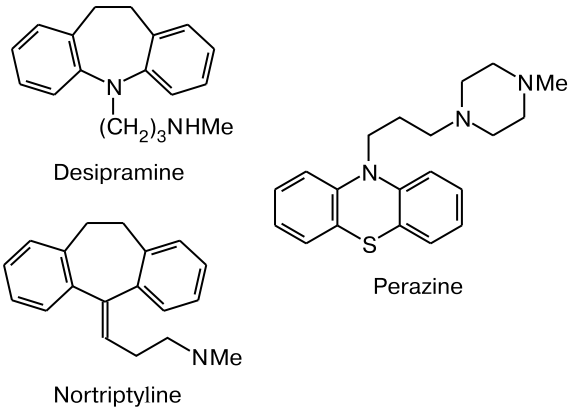
Scheme 52



69

78

79



Desipramine

Perazine

Nortriptyline

The N-oxidation at the aza groups of some derivatives of aromatic azaheterocycles was considered above.

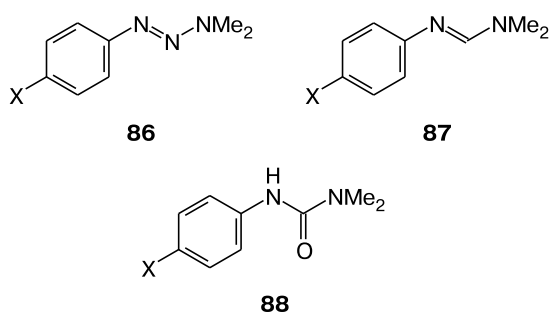
Of the drugs whose metabolism involves the N-oxidation of the dialkylamino group, let us note the antidepressants Imipramine (**69**), Zimeldine (**78**), and Amitriptyline (**79**) (Scheme 52), the monoamine oxidase inhibitor, the antihypertensive drug Pargyline (**80**), the local anesthetic Lidocaine (**81**), the anorexigenic drug Benzphetamine (**82**), the antiemetic antipsychotic tranquilizer Chlorpromazine (**83**), and the anti-Parkinson drug Selegiline (**84**) (Scheme 53).

It is interesting that imipramine N-oxide, which is the major metabolite of Imipramine, also has a strong antidepressant effect.

Compounds containing seven-membered rings are also oxidized at the nitrogen atom. For example, the known antihypertensive drug Guanethidine (**85**) undergoes N-oxidation at the tertiary nitrogen atom (catalyzed by FMO) (Scheme 54). It is interesting that the guanidine fragment in this case is resistant to oxidation.

It should be noted that flavin adenine dinucleotide (FAD)-containing monooxygenases catalyze the oxidation of different amines, for examples, of the antidepressants Desipramine and Nortriptyline and the antipsychotic Perazine.[27]

Many nitrogen-containing compounds, such as *N*,*N*-dimethyltriazenes (**86**), *N*,*N*′-dimethylformamidines (**87**), and *N*,*N*-dimethyl-*N*′-arylureas (**88**), undergo demethylation in the metabolism.

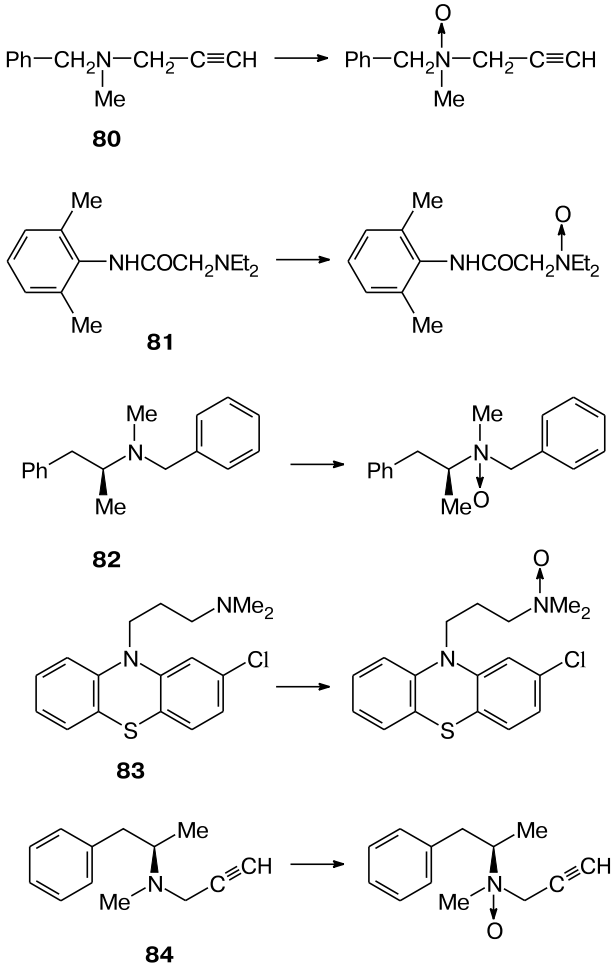


86

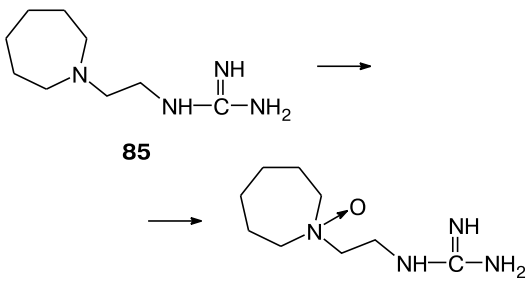
87

88

Scheme 53



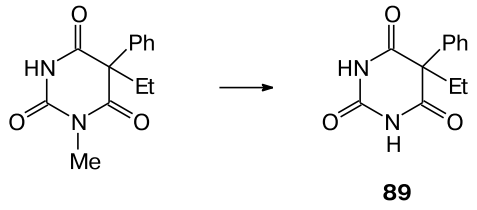
Scheme 54



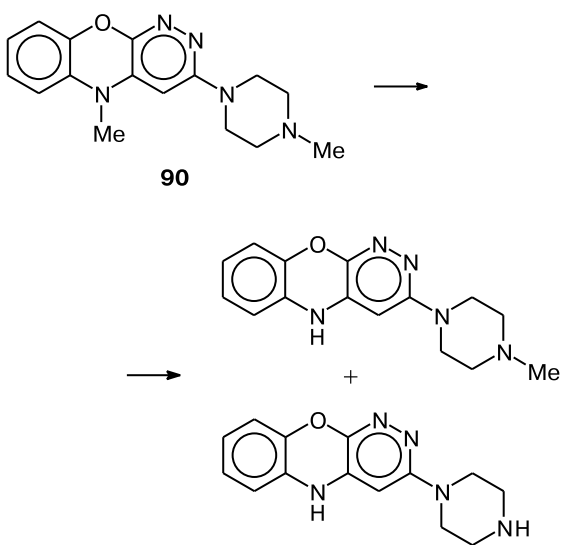
The transformation of 1-methylphenobarbital into phenobarbital (**89**) in the body (in the liver endoplasmic reticulum) (Scheme 55) is an example of the metabolic demethylation in the heterocyclic series (for drugs).

Asaphen (**90**) is metabolized to form demethyl and dedimethyl derivatives, the activity of the latter compounds being as high as that of Asaphen (Scheme 56).

Scheme 55

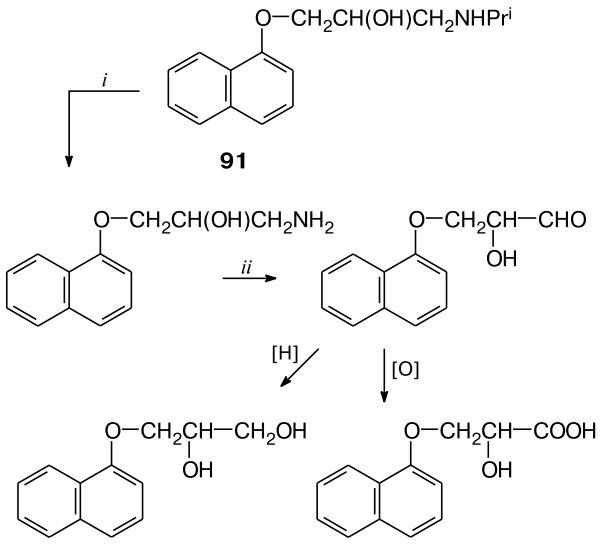


Scheme 56



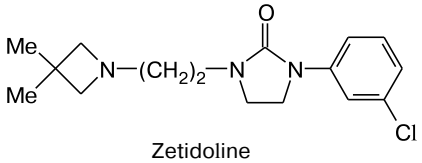
The dealkylation (*i*) is often accompanied by the oxidative deamination (*ii*), as was observed for the known β-adrenoblocker propranolol (**91**) (Scheme 57).

Scheme 57



i. Dealkylation; *ii*. deamination.

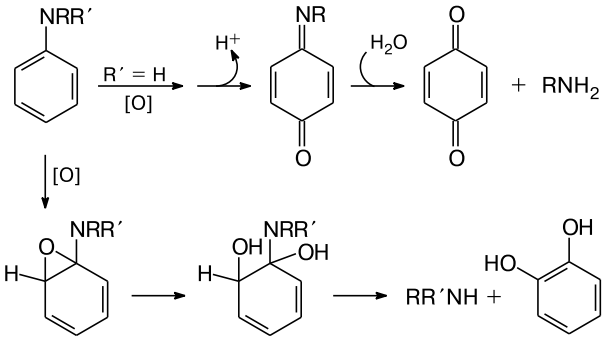
Of the drugs that undergo dearylation, let us mention the neuroleptic Zetidoline.[37]



Zetidoline

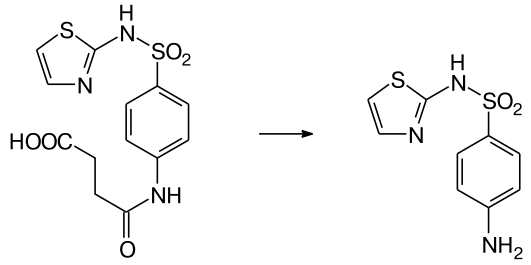
The hypothetical mechanism of oxidative dearylation,[2] which is a very rare process in organic synthesis, is presented in Scheme 58.

Scheme 58



Let us consider the metabolic deacylation.[1] There are cases when the intestinal metabolism is favorable for the oral administration. This relates, for example, to the sulfonamide antibiotic Succinylsulfathiazole (**92**) serving as a prodrug (Scheme 59). This compound is poorly absorbed through the intestinal walls and is easily hydrolyzed by intestinal enzymes to active sulfathiazole (**93**). This local hydrolysis produces a high effective concentration of the drug in the intestine, due to which it exerts a desired therapeutic effect in the treatment of intestinal infections.

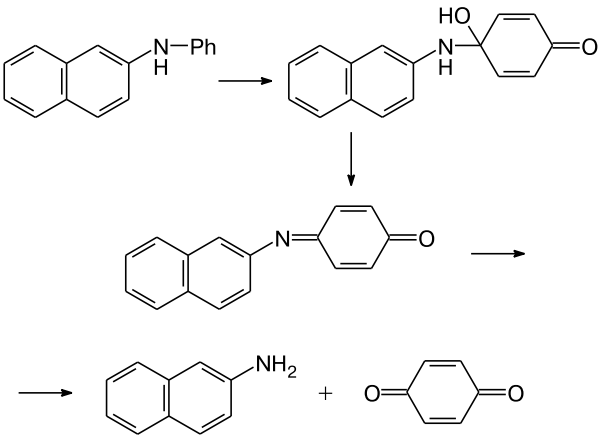
Scheme 59



92 **93**

As mentioned above, the dealkylation is a usual metabolic transformation of various N-alkyl derivatives. The transformations associated with the N-dearylation are more rare (see also the above-mentioned data on the neuroleptic Zetidoline).[37] For example, the hepatic microsomal metabolism of *N*-phenyl-2-naphthylamine affords the hydroxy metabolite, whose oxidation gives 2-naphtylamine and benzoquinone (Scheme 60).

Scheme 60



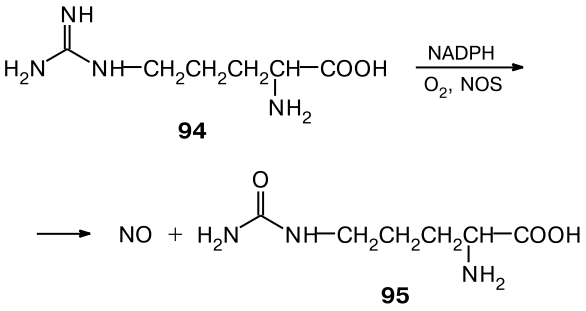
Generation of nitric oxide in the metabolism of heterocyclic derivatives

In the last 15 years, progress in biology caused fundamental changes in our knowledge about the functioning of various biological systems. It was found that such a low-molecular-weight compound as nitric oxide NO is one of the universal and necessary regulators of functions of cell metabolism. Unexpectedly, it appeared that the gas, which is toxic, whose molecule is moreover a free radical, and which is a short-lived compound that easily undergoes various chemical transformations, is continuously enzymatically produced in mammalians and has a key effect on various physiological and pathophysiological processes. Nitric oxide is involved in the regulation of the tone of blood vessels, inhibits the aggregation of thrombocytes and their adhesion to blood vessel walls, acts in the central and vegetative nervous system, and regulates the activity of the respiratory system, the gastrointestinal tract, and the urogenital system. Nitric oxide has a very broad spectrum of biological activities. It plays an important role in the neurotransmission, the immune regulation, and the body protection against bacterial damage. In 1992, the NO molecule was named "Molecule of the Year" in the journal Science. In 1998, three American scientists, R. Furchgott, L. Ignarro, and F. Murad, were awarded the Nobel Prize in Physiology and Medicine for their discoveries concerning nitric oxide as a signaling molecule in the cardiovascular system. The number of reviews and original publications devoted to the problem of NO greatly increased each year.[38–53]

This section deals with the analysis of the modern literature concerning the generation of nitric oxide in the metabolism of drugs belonging to azaheterocyclic compounds.

It is known that nitric oxide is generated in the body by NO synthase (NOS) as a result of the oxidation of the amino acid L-arginine (**94**), which is transformed into the ureido amino acid citrulline (**95**) (Scheme 61). The metabolism of arginine was considered in Chapter 1 of the monograph.[52]

Scheme 61



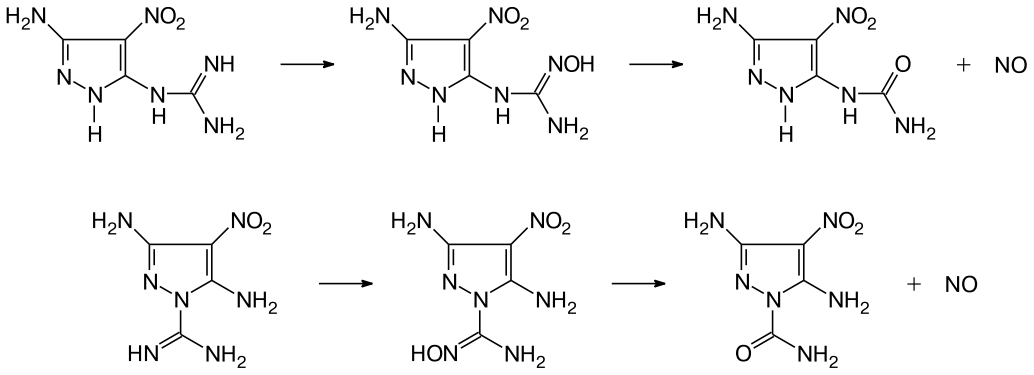
94 **95**

For various drugs, it was established that they release nitric oxide *in vitro* and many of them *in vivo*. For some of these agents, such as nitrates, *S*-nitrosothiols, sodium nitroprusside, and molsidomine, the major action is directly determined by the ability to generate NO.[52] For other agents, the generation of NO is apparently a minor function, and the elucidation of their role in the biological action calls for a serious study.

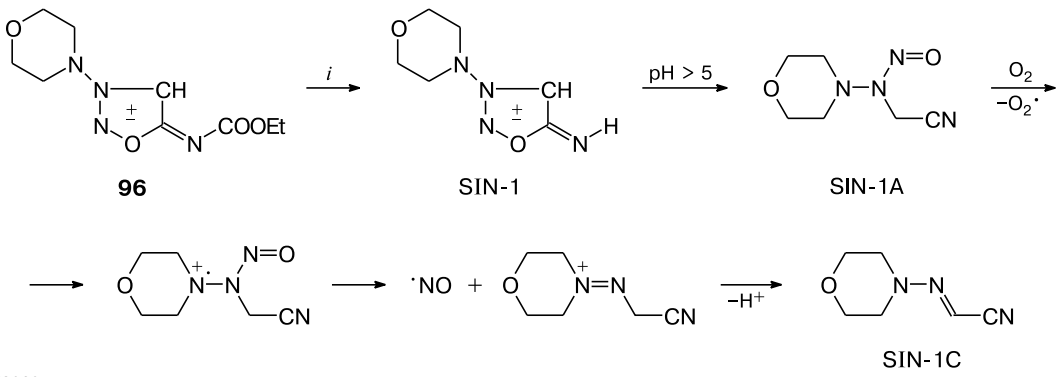
The problems of the generation of nitric oxide from exogenous compounds are related to the recent investigation on the ability of pyrazole derivatives containing the guanidine or amidine groups as substituents to undergo transformations accompanied by the release of a nitric oxide molecule during oxidation.[54] The oxidation by potassium ferricyanide was carried out in an alkaline medium, and the formation of the nitroprusside anion was detected by polarography. Apparently, the NO release occurs by the usual mechanism associated with the N-hydroxylation in the first step (Scheme 62).

As an example, I give the scheme of metabolism and degradation of the known anti-anginal drug Molsidomine (**96**)[55–58] whose biological action was established to be associated with its ability to release nitric oxide (Scheme 63).

Scheme 62



Scheme 63



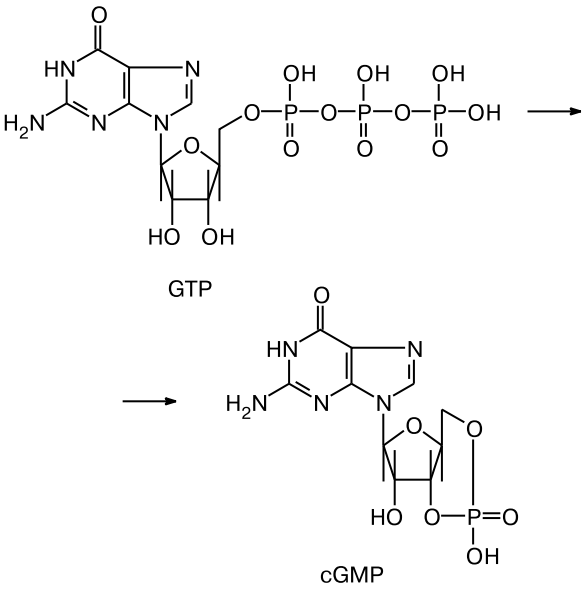
96 SIN-1 SIN-1A SIN-1C

i. Liver esterases.

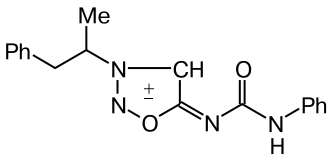
It was found that the decomposition of the intermediate SIN-1A (unlike that of SIN-1) is the pH-independent process and occurs with the involvement of oxygen. Moreover, there is the correlation between the oxygen consumption and the generation of nitric oxide in buffered solutions of SIN-1. It is essential that it is NO rather than HNO that is generated along with SIN-1C from SIN-1A. The intermediate SIN-1A is an activator of soluble guanylate cyclase (sGC) and increases the intracellular concentration of cyclic guanosine monophosphate (cGMP) generated from guanosine triphosphate (GTP) and, correspondingly, enhances its effects, *viz.*, the vasodilation, inhibition of thrombocyte aggregation, neurotransmission, and immune response regulation, which are typical of nitric oxide donors.

However, in spite of the diversity of biological effects caused by the NO release and different types of systems influenced by this regulator of metabolism, sGC located in vascular smooth muscle walls is the most important physiological target of nitric oxide in the body.[43] The functioning of guanylate cyclase is directly related to the physiological effects of NO, such as its antihypertensive and anti-aggregation properties (Scheme 64).

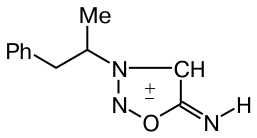
Scheme 64



The transformation of Molsidomine (**96**) accompanied by the NO release affords the superoxide radical anion, resulting in the peroxynitrite effects of SIN-1, such as the low-density lipoprotein oxidation, the deoxyribose degradation, the inhibition of glyceraldehyde-3-phosphate dehydrogenase, and the cytotoxic action. Scheme 63 presents the enzymatic degradation of Molsidomine (**96**). However, the non-enzymatic degradation of the drug is also possible. Thus, under physiological and more alkaline conditions, SIN-1 undergoes the more rapid non-enzymatic ring cleavage to form SIN-1A, which is relatively stable at pH 7.4 under anaerobic conditions in light-protected solutions. At the same time, even traces of oxygens promote further transformations up to the formation of *N*-morpholinoiminoacetonitrile (SIN-1C). It is essential that the visible-light irradiation leads to a substantial increase in the rate of oxygen-dependent nitric oxide release from SIN-1. Based on these data, the pathways of the possible biotransformation of the mesoionic psychotropic drugs Sydnocarb (**97**) and Sydnophen (**98**) were considered.[59] Both these drugs contain the β-phenylalkylamino group.



Sydnocarb



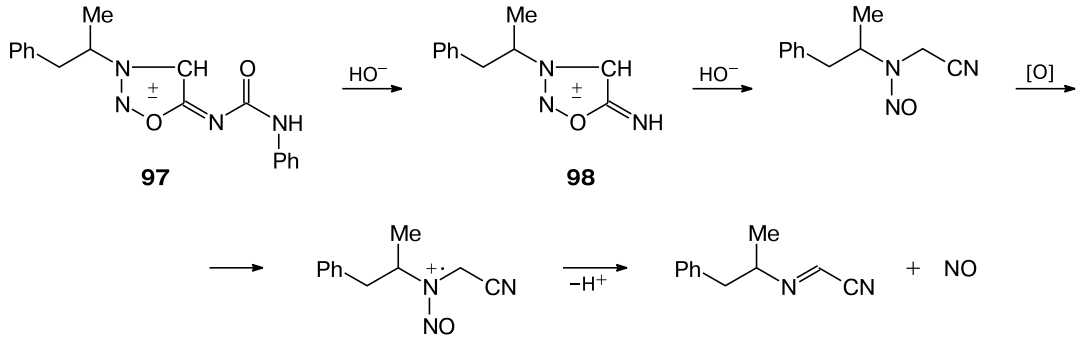
Sydnophen

The mechanisms of action of these compounds adopted in the pharmacological literature are associated with the indirect sympathomimetic effect consisting of the following two components: the release of the mediator noradrenaline from its neuron depot and the inhibition of its reuptake. It is well known that Sydnophen (**98**) is an effective monoamine oxidase inhibitor, which is responsible for its antidepressant action.

The effective psychostimulant Sydnocarb (**97**) is an indirect sympathomimetic agent. However, the pronounced structural similarity of these sydnonimine derivatives and Molsidomine (**96**) serving as a nitric oxide donor suggests that both Sydnophen and Sydnocarb, by analogy with Molsidomine,[60] can generate NO in the living body (Scheme 65).

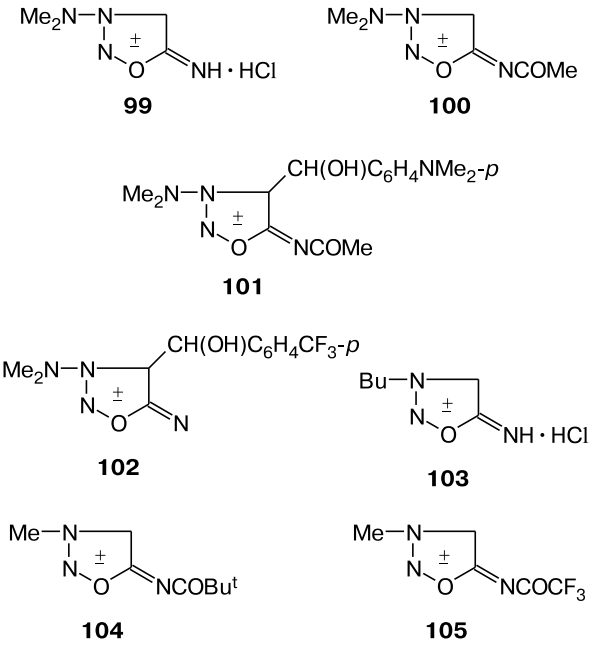
The polarographic study of the hydrolysis of Sydnocarb and Sydnophen in a phosphate buffer, pH 8.2, showed that the transformations presented in Scheme 65 afford *N*-nitroso-*N*-phenylisopropylaminoacetonitrile, whose oxidation by potassium ferricyanide gives nitric oxide. It should be noted that the oxidative step is the necessary condition for the nitric oxide release, because the NO generation is not observed under anaerobic conditions. In addition, the hydrolysis of the phenylcarbamoyl fragment in the first step is required for Sydnocarb. Therefore, the metabolic chain of the NO donor Molsidomine (**96**) is

Scheme 65



modeled *in vitro*, and hence the nitric oxide release in the body can occur when these drugs are used. However, there is no direct evidence of the significance of this process for the manifestation of the pharmacological activity of these mesoionic agents. The estimation of the contribution of released NO to the mechanism of psychotropic activity of Sydnocarb and Sydnophen calls for special biochemical and pharmacological research.

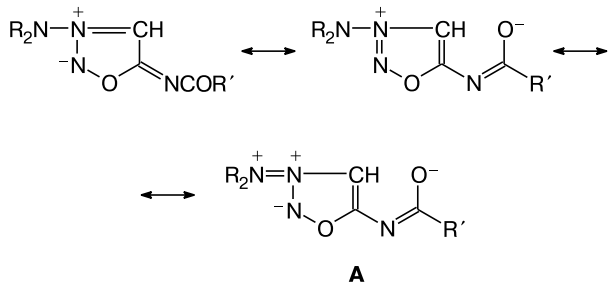
The investigation[61] also considered the NO donor ability of sydnonimines. Table 1 gives the yields of nitric oxide generated by the oxidation of sydnonimines **96** and **98—105** by atmospheric oxygen in an alkaline medium (0.1 *M* NaOH) at 80 °C for 30 min.



These sydnonimines are clearly divided into two groups: compounds bearing amino-containing substituents at position 3 of the heterocycle (**96** and **99—102**) and compounds bearing alkyl substituents at this position (**103—105**). The properties of the sydnonimines were compared with those of Molsidomine (**96**) and Sydnophen (**98**).

A comparison of the results, for example, for compounds **99** and **104** shows that the degradation of the former compound affords nitric oxide in an amount, which is more than an order of magnitude larger compared to the latter compound. It should be emphasized that, unlike usual amino-substituted aromatic compounds, in which amino groups act as strong electron donors, the amino substituent in sydnonimines at position 3 cannot be involved in the donor conjugation. Actually, the structure **A** does not make a substantial contribution to the resonance hybrid (Scheme 66).

Scheme 66



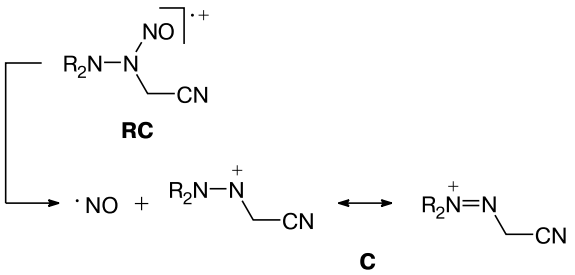
Hence, it seems possible that the first hydrolytic step resulting in the elimination of the *N*-phenylcarbamoyl

Table 1. Yields of nitric oxide in the oxidation of sydnonimines **96** and **98—105**

Compound	Yield of NO (%)
96	14±2
98	1±0.2
99	31±2
100	19±2
101	22±2
102	22±2
103	0.7±0.2
104	0.7±0.2
105	3.6±0.2

group under the action of the hydroxide anion occurs more easily in the presence of the dialkyl substituent, in particular, of the dimethylamino group, compared to compounds containing the electron-releasing alkyl substituent. However, it should be noted that this effect is not dominant. Otherwise, it cannot be explained why the yields of NO for compounds containing no *N-exo*-acyl groups (for example, **99** and **98** or **103**) are also substantially different. Apparently, the transformation of the radical cation **RC** into the cation **C** and nitric oxide plays the major role in this difference. In the cation **C**, the situation is radically different compared to the initial molecule, and the dialkylamino groups in the cation **C** are strong electron donors (Scheme 67).

Scheme 67



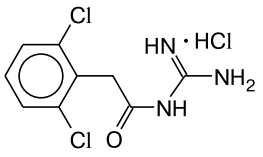
If assuming that the transition state of the process **RC** → **C** is similar to the final compound **C**, this stabilization should lead to a sharp decrease in the activation energy of the process and, correspondingly, to an increase in the degradation rate and, consequently, to a decrease in the contribution of side (with respect to the NO generation) reactions, such as the formation of nitroxide and the known recyclization of sydnonimines into oxotriazoles.

The results obtained in the study[61] are in good agreement with the preliminary data on the activation of the enzyme soluble guanylate cyclase (sGC) by these compounds. This enzyme catalyzes the formation of the secondary mediator, cyclic guanosine monophosphate, from guanosine triphosphate. As mentioned above, the investigation of the action of various drugs on sGC is a convenient and reliable test for the validation of the ability of the compounds under study to release nitric oxide, because the non-heme mechanism of activation of sGC is very rare. In the case under consideration, the activation of sGC by the first group of compounds is substantially higher than that caused by sydnonimines of the second group.

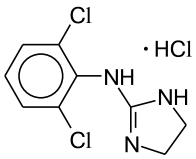
The results of the study[61] suggest that the most effective nitric oxide donors of this series should be searched for among compounds containing amino substituents at position 3 of the sydnonimine ring. This can be a rational approach to the search for new biologically active compounds.

Another direction of the search for nitric oxide donors among known drugs is based on the investigation of compounds containing the guanidine fragment. This approach seems to be reasonable because the guanidine NH group of L-arginine is responsible for the generation of nitric oxide under physiological conditions.

Guanfacine (**106**) and Clonidine (**107**) were chosen for studying in the first step.[52,59]



106

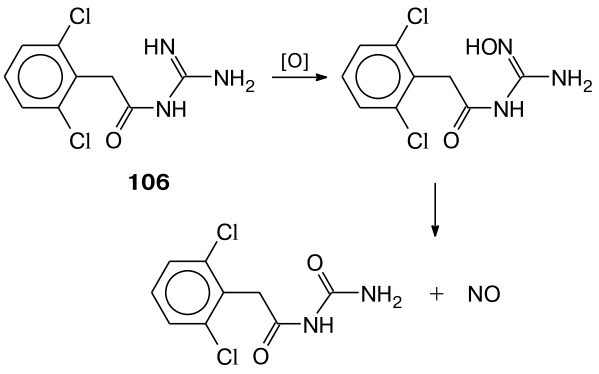


107

These drugs are stimulants of α_2-adrenoreceptors in vasomotor centers, decrease the sympathetic outflow from the central nervous system, and reduce the release of the mediator noradrenaline from nerve endings, resulting finally in the hypotensive effect. Both these drugs contain the guanidine fragment, thus being able to serve as substrates of the oxidative enzymes NO synthases. Actually, the polarographic study showed[52] that the oxidation of these compounds can lead to the elimination of nitric oxide. It should be noted that the activity of Guanfacine as an NO donor is higher than that of Clonidine containing the cyclic guanidine fragment and is somewhat lower than that of L-arginine (the main source of NO in the body). Under the conditions in which the oxidation was performed,[52] all the compounds under study, including arginine, produce nitric oxide.

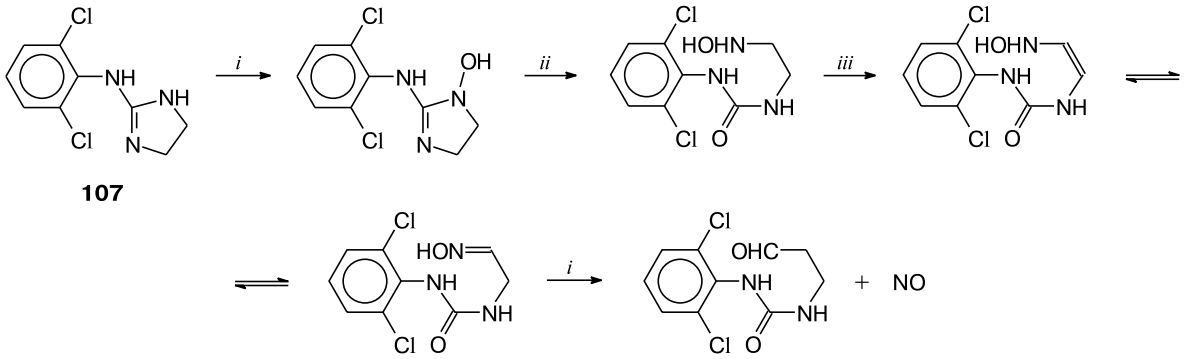
It was also found that Guanfacine (**106**) and Clonidine (**107**) cause the drug concentration-dependent activation of soluble guanylate cyclase. It was hypothesized that the oxidation of Guanfacine containing the open guanidine fragment occurs by the same mechanism as the oxidation of L-arginine (Scheme 68).

Scheme 68



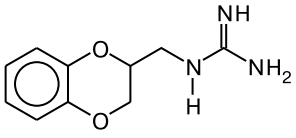
In the case of Clonidine (**107**), the situation is complicated by the cyclic imidazoline system, and the hypothetical scheme of oxidation of this drug is different (Scheme 69).

Scheme 69

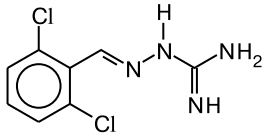


i. Oxidation. *ii*. Hydrolysis. *iii*. Dehydrogenation.

The dehydrogenation and oxidation of the resulting oxime (according to the scheme known for oximes) are the key steps. An attempt was made to accelerate the dehydrogenation by adding NAD^+, and it was shown that in the presence of this coenzyme, which can be involved in redox processes accompanied by the hydride ion transfer, the activation of soluble guanylate cyclase (sGC) is relatively sharply increased under the action of Clonidine (**107**). To a certain extent this fact confirms the validity of the above-considered scheme. Among drugs containing the guanidine fragment, there are the antihypertensive agents Guanoxan and Guanabenz.



Guanoxan

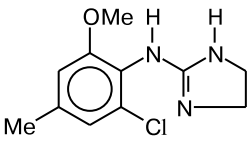


Guanabenz

It was found[52] that the treatment of Guanfacine (**106**), Guanoxan, and Guanabenz with the oxidizing agent sodium hypochlorite (10^{-3} mol L^{-1}) at 60 °C for 30 min at pH 9.2—11 affords nitric oxide in yields of 2—3, 4—6, and 4—6%, respectively.

It is important that both Guanoxan and Guanabenz activate soluble guanylate cyclase, the effect being significant particularly in the case of Guanoxan. This is a considerable evidence for the *in vivo* NO-donor ability of these drugs.

Moxonidine containing (like Clonidine) the cyclic guanidine (imidazoline) fragment is yet another agent attracting attention as a possible nitric oxide donor.[52]

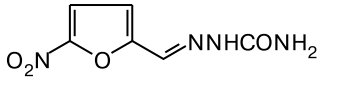


Moxonidine

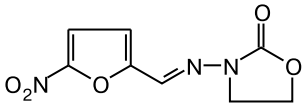
This interesting long-lasting antihypertensive agent is the imidazoline receptor agonist. The oxidation of Moxonidine by sodium hypochlorite under the same conditions as those used for Guanoxan and other related drugs (see above) produces NO in 2—3% yield. Moxonidine, as it is typical of NO donors, exhibits the properties of a stimulant of guanylate cyclase. As in the case of Clofelin, the addition of NAD^+ sometimes increases the level of activation of this enzyme, although this effect is less pronounced for Moxonidine and is observed not at all concentrations.

Known antimicrobial agents of the nitrofuran series are of interest in terms of this problem. Nitrofurans are effective with respect to Gram-positive and Gram-negative bacteria. Since it is known that the furan ring can be cleaved by various nucleophilic agents, it was suggested that, under reducing condition, nitrofuran derivatives can undergo dearomatization, which, at least partially, should result in the formation of compounds capable of releasing nitric oxide.[62]

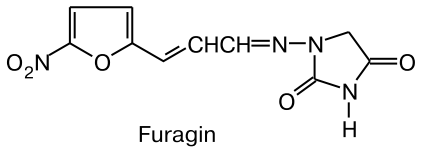
The behavior of a number of drugs of the nitrofuran series, such as Furacillin, Furagin, and Furazolidone, under reducing conditions was studied.



Furacillin



Furazolidone



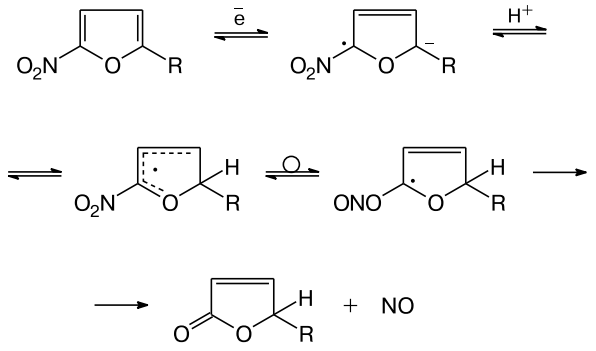
Furagin

The heating of solutions of these compounds (at concentrations of 10^{-4} and $2 \cdot 10^{-4}$ mol L^{-1}) with potassium

ferrocyanide ($c = 10^{-3}$ mol L^{-1}) at 60 °C and pH 5 resulted in the formation of the nitroprusside anion (polarographic data), which is evidence that nitric oxide is generated under reducing conditions. The second type of reduction of nitrofuran derivatives is based on the reduction by ascorbic acid (pH 6.5) followed by the addition of potassium ferrocyanide and also affords nitroprusside as a result of the nitric oxide release under these conditions.

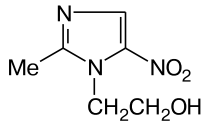
There are published data that the radical anions generated by the reduction of 5-nitrofurans can undergo the nitro→nitroether rearrangement. Based on this fact, the mechanism was proposed[62] for the generation of nitric oxide upon the degradation of drugs of the nitrofuran series (Scheme 70).

Scheme 70

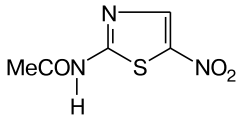


As mentioned above, many nitric oxide effects are associated with the formation of peroxynitrite. Presumably, the antibacterial action of the latter compound is determined by the inhibition of the mitochondrial electron transport chain, resulting in the inhibition of cellular respiration in microorganisms. Consequently, it cannot be ruled out that the ability of the above-considered drugs to generate nitric oxide is one of the factors responsible for their high antimicrobial activity.

Finally, let us consider two drugs derived from nitroheterocycles, like the above-considered nitrofurans. These are the known antiprotozoal drugs Metronidazole (**108**) and Nitazole (**109**).



108



109

Metronidazole, 1-(2-oxyethyl)-2-methyl-5-nitroimidazole, has a broad spectrum of chemotherapeutic activities against anaerobic protozoa, Gram-negative bacteria, and some Gram-positive bacteria. As was shown by electrochemical methods and ESR, Metronidazole easily gives the one-electron reduction product of the nitro group (the radical anion), which can cleave DNA, RNA, and other vital cellular macromolecules, and it is this radical anion that is apparently responsible for the biological activity of the drug.[63] However, the mechanism of action of Metronidazole remains an open question. For example, it was suggested that the corresponding hydroxylamine derivative obtained by the reduction is the active form of the drug.[64] In this context, it should be noted that the nitro group can be eliminated in the reduction of Metronidazole.[63–65]

For instance, it is known that the electrolysis of Metronidazole affords nitrite ions in high yield;[65] the reduction of the drug in solution in the presence of thiols and divalent iron ions gives complexes containing iron ions, molecules of thio derivatives, and nitric oxide.[66]

It should be emphasized that N-substituted 5-nitro-2-styrylimidazoles in alkaline media (pH > 10) undergo the cleavage accompanied by the opening of the imidazole ring and elimination of the nitro group as the nitrite anion.[67,68] Based on the above-considered data, it was suggested[69] that the nitric oxide release is the possible pathway of the biotransformation of Metronidazole and, apparently, this fact plays an important role in the mechanism of biological action of this drug.

Polarographic measurements showed that the alkaline hydrolysis of this drug gives nitric oxide. The rate constant of hydrolysis of Metronidazole in 0.01 *M* NaOH at 80 °C is $1.6 \cdot 10^{-3}$ min^{-1}. In 0.1 *M* NaOH, the elimination of the nitro group from Metronidazole under these conditions goes almost to completion within 30 min.

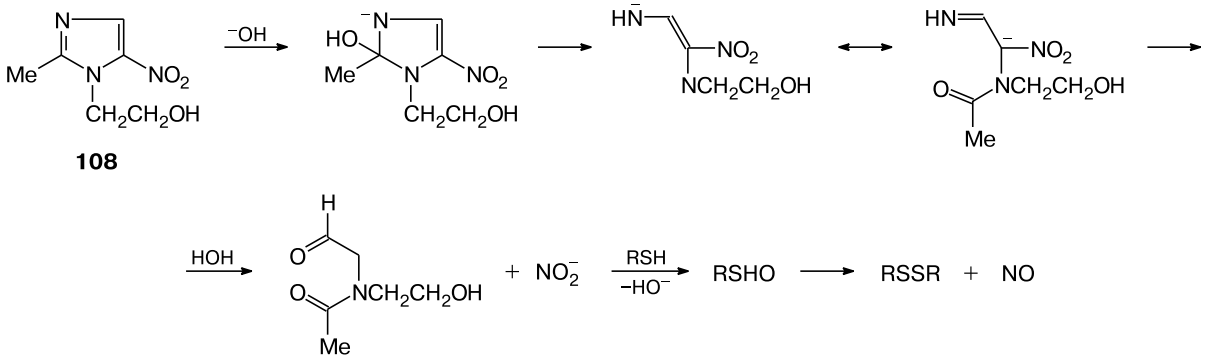
The nitric oxide release in the degradation of Metronidazole and in biological systems (human blood) is confirmed by the activation of the enzyme sGC by this drug. The degree of activation depends, as it is typical of exogenous NO donors, on the concentration of Metronidazole.

The discovered NO-donor properties of Metronidazole have stimulated the authors of the publication[69] to estimate the influence of this drug on such hemodynamic parameters, as the heartbeat rate and the systemic blood pressure in experiments on animals and to compare these results with the data for isosorbide mononitrate, which is a typical NO donor. It appeared that the effects of Metronidazole are very similar to those of isosorbide-5-mononitrate.

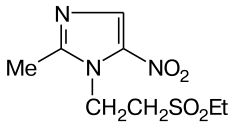
The scheme of the generation of nitric oxide from Metronidazole (**108**) under alkaline conditions involves the nucleophilic attack of the hydroxide anion on position 2 of the imidazole moiety of the molecule followed by the cleavage of the heterocycle and the subsequent elimination of the nitrite anion (Scheme 71).

However, it cannot be ruled out that this process involves the nitro→O-nitroso rearrangement similar to that suggested for nitrofuran derivatives (see above). As for the possibility of the generation of nitric oxide as a result of reduction, the consideration of the scheme of this process requires additional data.

Scheme 71



Finally, there are similar data on the drug Nitazole (**109**). Under the conditions described for Metronidazole, the latter drug also releases NO and activates soluble guanylate cyclase.[70] The problems associated with the metabolism of the known antiprotozoal drug Metronidazole (**108**) were considered above in detail. In addition, it is reasonable to consider metabolic transformations of another chemotherapeutic drug of this series, *viz.*, Tinidazole, 1-(2-ethylsulfonyl)ethyl-2-methyl-5-nitroimidazole (**110**), belonging to 5(4)-nitroimidazole derivatives.[71] It is known that nitroimidazoles easily produce nitro radical anions as one-electron reduction products. Nowadays, it is assumed that this fact lies at the basis of the mechanism of their action.[72]



110

Recently, the alkaline hydrolysis of Metronidazole has been found to result in the release of NO_2^-.[69] As follows from the consideration of the mechanism of this process, the possible alkaline hydrolysis products are identical to the compounds found upon the administration of Metronidazole into biological systems, for example, upon the incubation of the drug with xanthine oxidase. The suggestion about the NO-donor properties of this drug was confirmed by biochemical and pharmacological experiments. This drug causes the activation of the enzyme sGC, which is characteristic of NO donors. In experiments on animals, this drug has an effect on the heartbeat rate and the systemic arterial pressure. It should be noted that changes in these parameters caused by the administration of Metronidazole are similar to the changes observed upon the introduction of isosorbite-5-mononitrate seving as the typical NO donor.[69]

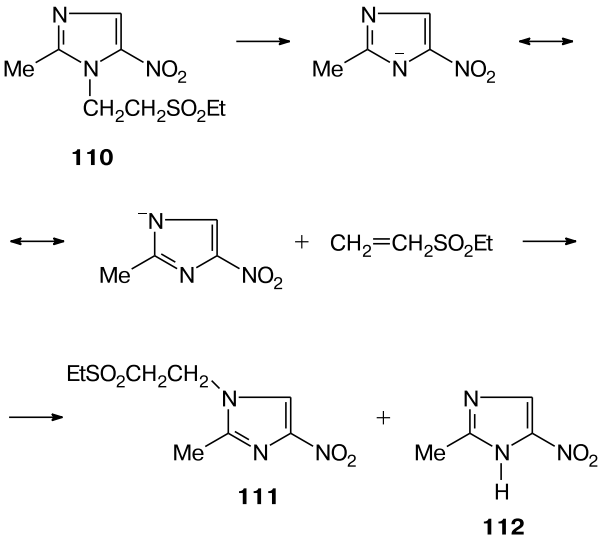
Since Tinidazole is a close analog to Metronidazole in both the chemical structure and the spectrum of chemotherapeutic activities, these drugs presumably have a similar mechanism of action and, consequently, possess similar NO-donor properties.

The hydrolytic transformations of Tinidazole were investigated by direct-current polarography, and the possible elimination of the nitro group was monitored by the Griess reaction. According to the results of the study,[69] Metronidazole is almost completely decomposed at 80 °C during 30 min with the elimination of nitrite anions in virtually quantitative yield. However, attempts to obtain a similar result for Tinidazole failed. Unlike Metronidazole, Tinidazole releases at most 8% of nitrite under these drastic conditions. These data are seemingly contradictory to the results of investigation on the stability of these drugs. Thus, at pH 11 and 80 °C, the rate constant of hydrolysis of Metronidazole is 150 times lower than that of Tinidazole.[73] Nevertheless, the polarograms obtained both in alkaline solutions of Metronidazole and in alkaline solutions of Tinidazole after heating at 80 °C for 30 min confirm the degradation of both drugs. It should be noted that in the case of Metronidazole, no electrochemically active compounds were found in solution. By contrast, Tinidazole is characterized by a wave with the limiting current, which is somewhat higher than that of the polarographic wave in the initial solution. However, the half-wave potential is substantially shifted to negative potentials compared to the initial value, *i.e.*, a new compound, which also contains the nitro group, is apparently formed. Metronidazole is more stable in alkaline solutions than Tinidazole and, unlike the latter compound, does not undergo irreversible changes in these solutions at moderate temperature. This is evident from a comparison of the polarograms, which were obtained in an acidic solution immediately after dissolution of the weighted sample in this solution and in a solution of the same composition but after the storage for 24 h at alkaline pH followed by acidification. In the case of Metronidazole, no changes were observed, whereas a new compound was detected in the case of Tinidazole.

The observed differences can be explained based on the chemical behavior of imidazole derivatives containing the 2-(alkylsulfonyl)ethyl group at position 1 described in the literature. It was shown that in alkaline solutions, compounds of this group are readily transformed into two products, *viz.*, isomeric 4-nitroimidazole (**111**) and 2-methyl-

5-nitroimidazole (**112**), with the latter compound predominating at a high alkali concentration, whereas at a low concentration (for example, in the presence of sodium bicarbonate at a concentration of 4 g L^{-1}) 4-nitroimidazole is produced in virtually quantitative yield (Scheme 72).

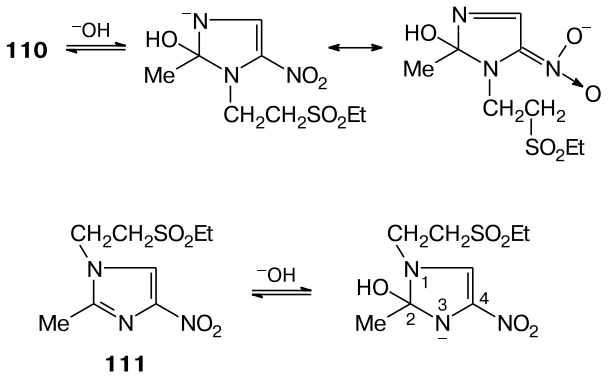
Scheme 72



Therefore, it seems evident that under hydrolytic conditions (in a strongly alkaline medium), 5-nitro compounds can undergo a transformation accompanied by the nitric oxide release (Metronidazole and Tinidazole), but this transformation is not typical of their 4-nitro isomers. This phenomenon can be interpreted as follows. First, two bulky groups (the nitro and alkylsulfonylethyl groups) in Tinidazole, as opposed to 2-methyl-5-nitroimidazole (**112**), are closely spaced, which sterically destabilizes the molecule and facilitates its degradation. Second, if assuming that the attack of the hydroxide anion, which precedes the cleavage of the imidazole ring of Tinidazole accompanied by the NO release,[69] occurs on position 2 and it this step that is rate-limiting, there are substantial differences between Tinidazole and 2-methyl-5-nitroimidazole (**112**) (Scheme 73). In the case of 5-nitro-substituted derivatives, the resulting anion is efficiently stabilized by the conjugation, by contrast to 4-nitro derivatives. Correspondingly, the rate of addition of the hydroxide anion is substantially higher for the 5-nitro derivatives.

Hence, in the case of Tinidazole, the processes in an alkaline medium resulting in the elimination of the nitro group (similar to those observed in solutions of Metronidazole) compete with the processes giving 4-nitroimidazole derivatives, which do not eliminate the nitro group under these conditions. The results of polarographic measurements and the analysis of hydrolyzed solutions of the drug for the presence of nitrite anions confirm this hypothesis.

Scheme 73



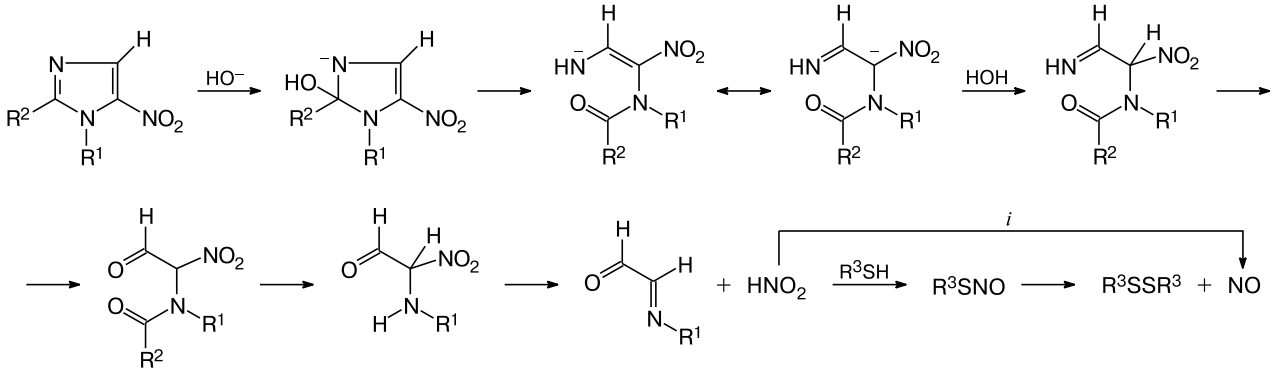
The use of more severe conditions of hydrolysis for Tinidazole, as opposed to Metronidazole, does not lead to an increase in the yield of nitrite anions. This can be explained in terms of the above-described scheme. According to the available data,[73] the elimination of the nitro group from the imidazole ring in an alkaline solution can occur as a result of the nucleophilic attack. An increase in the basicity, though leads to an increase in the concentration of the nucleophile (OH^-), accelerates, as mentioned above, the formation of the 4-nitro isomer of Tinidazole, which is thermodynamically more stable than the starting drug. Therefore, the elimination of the nitro group of Tinidazole is facilitated by the relatively mild conditions, under which processes resulting in the rearrangement occur more slowly.

Under mild conditions, Tinidazole more readily eliminates the nitrite anion compared to Metronidazole. The latter compound does not undergo this process at pH lower 10; in a buffer solution with pH 10 at 80 °C, Metronidazole gives nitrite anions in 2—3% yield within 1.5 h. By contrast, Tinidazole produces nitrite anions in comparable amounts in buffer solutions with lower pH 8—9 already at 37 °C.

Schemes were proposed for the alkaline hydrolysis involving the nucleophilic attack on positions 4 or 2 of the imidazole ring of Metronidazole and the elimination of the nitro group after the ring opening.[69] If the addition of the hydroxide anion occurs at position 2, which, as mentioned above, is in good agreement with the data on the difference in the degradation of Metronidazole and Tinidazole in an alkaline medium, the following general Scheme 74 can be suggested.

For Metronidazole ($R^1 = CH_2CH_2OH$, $R^2 = Me$), the hydrolysis according to this scheme can easily be predicted to form, in addition to nitrite, such compounds as acetamide, ethanolamine, and glycolic acid. It is noteworthy that these products were found among the compounds produced in the interaction of Metronidazole with biological systems of different complexity: after the anaerobic

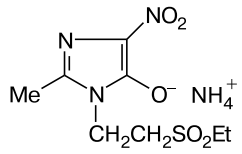
Scheme 74



i. Xanthine oxidase.

incubation with xanthine oxidase, the rat cecum content, and the culture *Clostridium perfringens*, as well as in urea of unsterile rats injected with Metronidazole. The most complete data were obtained for the incubation of Metronidazole with xanthine oxidase.[74]

For Tinidazole, the fact that the chemical and metabolic transformations are competitive can be illustrated by the presence of the 4-nitroimidazole derivative, *viz.*, ammonium 1-(2-ethylsulfonyl)ethyl-2-methyl-4-nitro-4,5-dihydro-1*H*-imidazol-5-olate (**113**), among metabolites.[75]



113

Therefore, in terms of the concept that the biotransformation of drugs can be modeled by interactions of chemical reagents, it is concluded that the fragmentation of Tinidazole in the body can be accompanied by the NO release, and the action of the drug on anaerobic protozoa and bacteria is presumably associated, to a certain extent, with its NO-donor properties.

It was shown[75] that some C-nitro derivatives of pyrazole, imidazole, 1,2,4-triazole, and isoxazole can generate NO when heated with an alkali, and the efficiency of the nitric oxide release was related to the nature of the heterocycle and the number of nitro groups in the heterocycle.

Under the above-mentioned conditions, the elimination of the nitrite anion can occur by two main mechanisms (note that the nitrite anion serves as a marker of the generation of nitric oxide). It should be mentioned that, in addition to the NO synthase pathway, living organisms have an alternative nitric oxide source, *viz.*, the nitrite—nitrate—xanthine oxidase route. Xanthine oxidase can generate NO using nitrite ions as the substrate.[52]

The former mechanism of elimination of the nitro group involves the *ipso* substitution characterized by the formation of the corresponding σ complex followed by the elimination of NO_2^- and the nucleophilic attack on the anion (in the case under consideration, on the hydroxide anion) and accompanied by the cleavage of the aromatic heterocycle to form the less stabilized system, which can be decomposed to give the nitrite anion.

Nitric oxide can alternatively be released through the cleavage of the heterocycle at a position different from the carbon atom bound to the nitro group and the dearomatization of the system followed by the nitric oxide release from the non-aromatic precursor.

A comparison of the aromaticity indices of the basic heterocycles, whose derivatives are considered in the present review, shows that the degree of aromaticity of the compounds under study decreases in the following series:

benzene > 1,2,4-triazole > pyrazole > imidazole > isoxazole.

It was shown[75] that the amount of nitrite anions generated by heating solutions of the mononitro derivatives in 0.1 *M* NaOH containing 10% of ethanol at 70 °C for 180 min depends only slightly on the nature of the basic heterocycle. Thus, the yield of nitric oxide was ~2% for nitropyrazole and nitroimidazole derivatives and was slightly higher (~3%) for nitrotriazole. As expected, the storage of the nitro derivative of isoxazole under these conditions led to a substantial degradation of the initial molecule, and the yield of NO_2^- was 26%.

The release of nitrite anions is studied by heating in an alkaline solution, *i.e.*, under conditions far from physiological. In the monograph,[2] the cleavage of heterocycles under hydrolytic conditions was discussed and it was mentioned that these processes can occur both under non-enzymatic conditions and under conditions of enzymatic catalysis. In many cases, the mechanism of hydrolysis of heterocycles in the body remains unknown, but, for example, for Tinidazole derivatives the ring opening was noted to be catalyzed by the enzyme L-5-oxoprolinase.

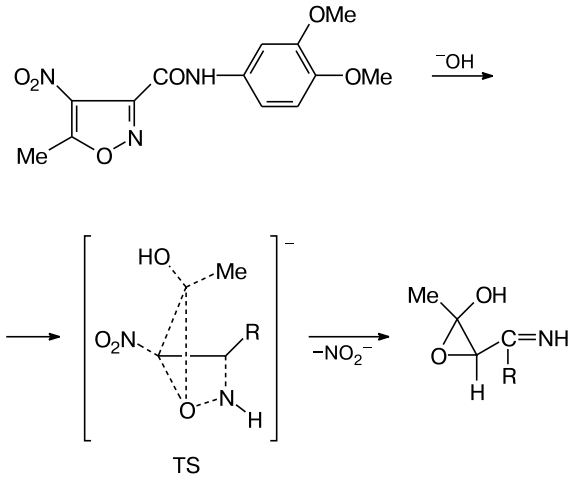
The hydrolytic transformation of the nitro derivatives under study is a model process, which was carried out under non-physiological conditions; however, it cannot be ruled out that the compounds under consideration can

serve as a source of NO in the body as well, because the rates of the processes and the yields of the final products under enzymatic catalysis increase manifold.

The fact that considerable amounts of nitrite anions were detected after the degradation of five-membered nitroheterocycles strongly suggests that these compounds can generate nitric oxide in living organisms as well. This is indirectly confirmed by the data on the activation of soluble guanylate cyclase by these compounds.

The kinetics of the nitric oxide release resulting from the hydrolysis of compounds was studied.[75] The thermodynamic parameters were calculated from the measured rate constants (k_{aver}) of these processes at different temperatures. Based on these parameters, suggestions were made about the reaction mechanism. Thus, Scheme 75 was proposed for the degradation of the nitro derivative of isoxazole.

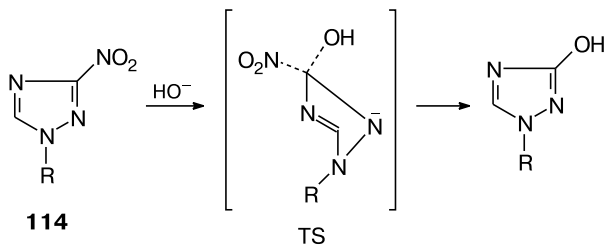
Scheme 75



TS is the transition state.

The very high negative value of the entropy of activation for 1-(*N*-*p*-ethylphenyl)carbamoylmethyl-3-nitro-1,2,4-triazole (**114**) is also evidence for the formation of the highly ordered transition state (Scheme 76).

Scheme 76



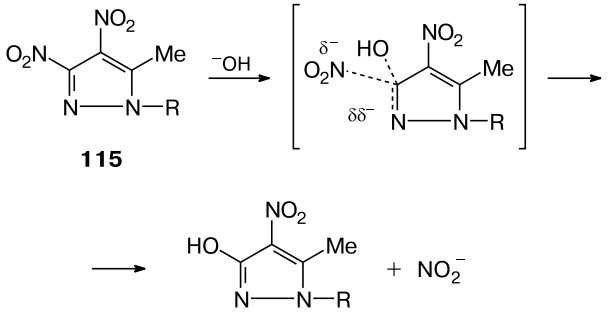
114

R = $CH_2CONHC_6H_4Et$-*p*

Evidently, the intensity of the interaction with nucleophilic reagents should sharply increase with increasing number of electron-withdrawing groups in the heterocycle. The elimination of the nitrite ion under nucleophilic attack on dinitro derivatives is more probable compared to the corresponding mononitro derivatives.

The results obtained in the study of the alkaline degradation of 1-(*p*-methoxyphenyl)carbamoylmethyl-5-methyl-3,4-dinitropyrazole (**115**) are interpreted as follows. The relatively high enthalpy of activation indicates that the bond cleavage in the transition state occurs to a great extent (Scheme 77) or, at least, the process is relatively deep. The moderate negative entropy of activation can be attributed to the same structural factors. Thus, the bond is almost cleaved and the steric parameters of the transition state are relatively similar to those of the initial state. As a result, the entropy of the transition state is similar to that of the initial state $\Delta S^{\#}$ and, correspondingly, is virtually equal to zero.

Scheme 77



115

R = $CH_2CONNC_6H_4OMe$-*p*

The behavior of dinitroimidazole differs dramatically from that of the above-considered pyrazole derivatives, which can in no way be explained by the difference in the nature of the substituents at position 1. First, the fast (~12 min) and quantitative elimination of the nitrite anion is observed for the imidazole derivative. Besides, the next step of the process is the much slower and evident, though not quantitative, continuation of the process associated with the degradation of the intermediate accompanied by the elimination of yet another nitro group in the form of the second nitrite anion.

It seems evident that the mechanism of the process under consideration is radically different from the mechanism of degradation of the pyrazole derivative. In the case of imidazole, the enthalpy of activation is substantially lower, whereas the entropy of activation is much more negative, *i.e.*, the situation is similar to that observed for the mononitroisoxazole derivative. It should be emphasized once again that the imidazole ring is less aromatic than the triazole and pyrazole rings. To interpret the results obtained for the imidazole derivative, the authors[75]

were based on the fact that in the case of the attack of the hydroxide anion, the cleavage of the imidazole ring occurs in the step that limits the rate of elimination of the nitrite anion (Scheme 78).

Finally, let us note that the above-mentioned schemes are to a large extent hypothetical and are confirmed only by the results of kinetic studies. The main result obtained in the study[75] is the experimental evidence that C-nitro derivatives (particularly, dinitro derivatives) of azoles exhibit significant NO-donor properties, which are determined by their structural characteristics and stability under nucleophilic attack. From this point of view, it is important that the above-considered compounds of this type and their analogs can release nitric oxide and are of considerable interest for studying their biological activity.

In this context, it is important to discuss the results of another recent research[76] on the transformations of (5-nitropyrimidin-4-yl)dialkyldithiocarbamates as new potential nitric oxide donors in mammalian organisms.

The investigation of nitro derivatives of pyrimidine containing the dithiocarbamoyl fragment in the *ortho* position with respect to the nitro group is one of the problems of considerable interest from both chemical and biological points of view. It should be noted that many compounds of the dialkyldithiocarbamate series exhibit considerable biological activities, such as fungicidal, insecticidal, herbicidal, anticholesterol, hypotensive, and antimicrobial activities, antifungal action, and antihelminthic activity; however, the mechanisms of these activities are poorly known. Hence, a search for biologically active compounds in a series of poorly studied nitroheteryl derivatives of dithiocarbamates is a topical problem.

Recently, it has been found that the thermal treatment of this type of compounds results in processes associated with the removal of the nitro group, the release being facilitated by the adjacent dithiocarbamoyl fragment.[77]

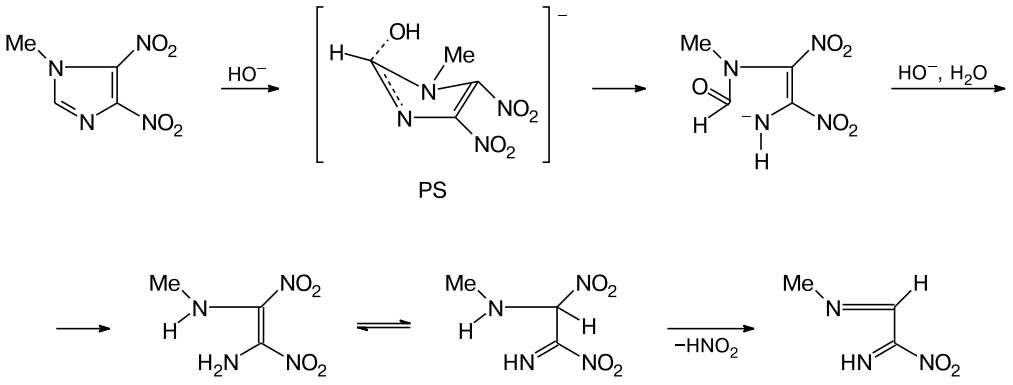
The research on the denitration of dithiocarbamoyl derivatives of nitropyrimidines under hydrolytic conditions revealed some characteristic features allowing 4-dialkyldithiocarbamoyl-5-nitropyrimidines to serve as nitric oxide donors. The investigation of the NO-donor ability of 4-dialkyldithiocarbamoyl-5-nitro-6-R-pyrimidines showed than most of these compounds undergo the degradation to give a nitrite ion upon heating in a buffer solution with nearly physiological pH (phosphate buffer, pH 6.86). It should be noted that the amount of NO_2^- that is released in the process depends on the nature of the substituent at position 6 of the pyrimidine ring. The largest amount of the detected nitrite anions is observed for compounds containing the phenoxy or methoxy group at position 6, whereas the replacement of these groups by stronger electron donors (amino substituents) substantially decreases its elimination.

In the synthetic part of the study,[77] the degradation of the compounds was shown to proceed by two main pathways to give disulfides and 4-dialkylamino-5-nitro-6-R-pyrimidine derivatives. Evidently, only the former pathway is accompanied by the elimination of the nitro group and, correspondingly, by the formation of the nitrite anion. Consequently, the nitric oxide release in living organisms should, apparently, be accompanied by the formation of disulfides.

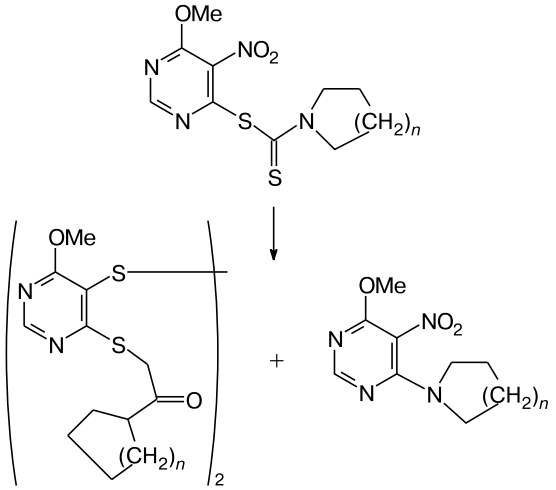
The reaction mixtures that are formed after heating in a phosphate buffer (pH = 6.86) were studied (^{1}H NMR) for the compounds containing an amine fragment with a different ring size in the dithiocarbamoyl moiety. It appeared that the pyrrolidine derivative gives a mixture of disulfide and 4-pyrrolidinopyrimidine in a ratio of 3 : 2 (a series of unidentified minor admixtures are produced); for the piperidine mixture, the ratio of the corresponding products is 9 : 1 (minor admixtures are present in an approximately the same amounts); the process with the involvement of the hexahydroazepine-substituted derivative affords only disulfide, the amounts of admixtures being insignificant (Scheme 79).

In studies of the processes under the above-mentioned conditions, the following intriguing features needing the interpretation were found.

Scheme 78



Scheme 79



n = 1—3

1. The replacement of the pyrrolidine fragment in 4-dialkyldithiocarbamoyl-5-nitro-6-R-pyrimidines successively by the piperidine and hexahydroazepine moieties leads to an increase in the amount of released nitric oxide, as was unambiguously confirmed.

2. In the case of compounds containing the methyl substituent instead of the proton at position 2 of the pyrimidine ring, the nitric oxide release upon the degradation dramatically decreases.

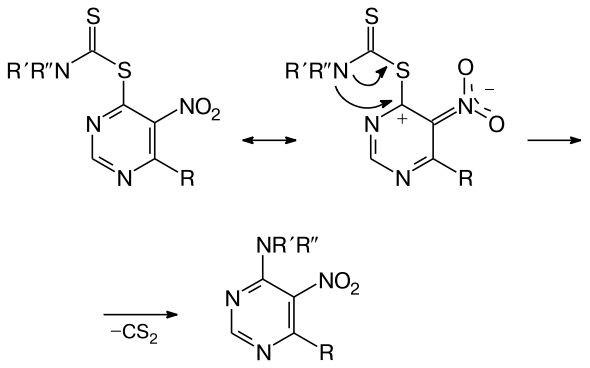
3. The elimination of the nitrite anion under hydrolytic conditions is observed only under aerobic conditions. Under argon, the amount of nitrite anions is within the detection limit.

To interpret the above-mentioned experimental data, the authors suggested the sequence of transformations given in Scheme 80.

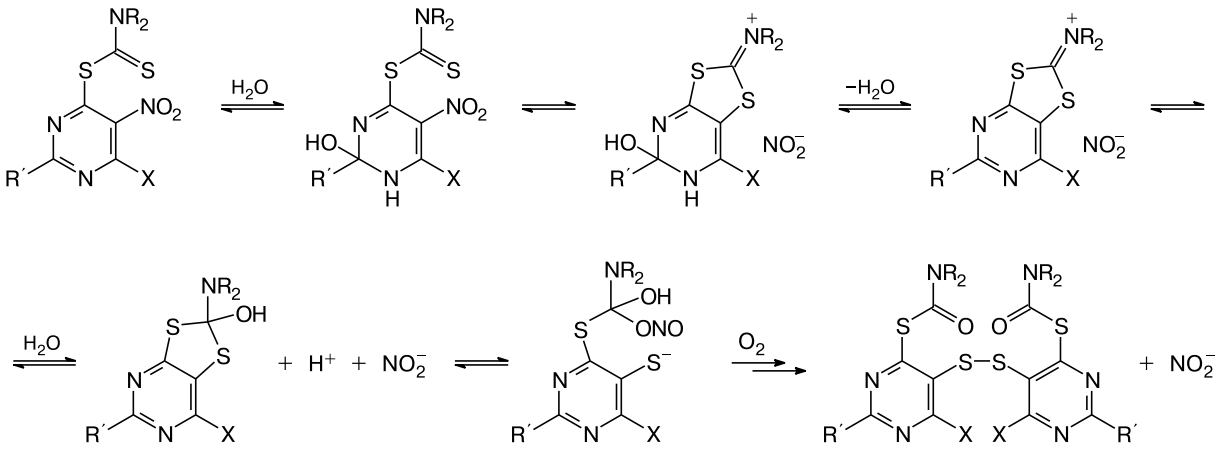
In principle, the formation of O-nitroso compounds can lead (apparently, after the oxidation step) to the elimination of NO^+, which, in turn, can cause also the nitrosation at the thiolate anionic moiety to form S-nitrosothiols. The latter are known[52] to be effective nitric oxide donors. These processes are reversible, and finally the system is stabilized by the subsequent irreversible oxidation giving rise to disulfide. The latter process requires the presence of oxygen in the system, which, as mentioned above, is the necessary condition for the formation of the nitrite ion in this reaction (evidently, the aerobic conditions are necessary for the processes presented in Schemes 7 and 8 as well).

The second process that occurs under the selected conditions affords 4-aminopyrimidine derivatives accompanied by the elimination of carbon disulfide. The latter was detected in the reaction mixture by gas-liquid chromatography. The scheme of this reaction is based on the nucleophilic attack of the nitrogen lone pair of the dithiocarbamate fragment on the carbon atom of the pyrimidine ring at position 4 (Scheme 81).

Scheme 81



Scheme 80



Based on the above-given schemes, the authors[76] explained also other non-obvious facts that characterize this process. Thus, the greatest steric crowding of the hexahydroazepine derivative leads to the destabilization and this dithiolone is more readily oxidized to disulfide compared to the pyrrolidine and piperidine compounds. A sharp decrease in the amount of nitrite ions formed by hydrolysis of compounds containing a substituent at position 2 of the pyrimidine ring is apparently attributed to the fact that the elimination of NO_2^- occurs only after the dearomatization of the pyrimidine ring through the addition of the hydroxide anion at position 2. This addition is hindered in the presence of the alkyl substituent instead of the proton at position 2, which decreases the efficiency of further processes, for example, for 2-methyl-substituted derivatives.

Therefore, under hydrolytic conditions, nitro derivatives of pyrimidyldithiocarbamates were found to be able to release the nitrite anion, which may be indicative of the ability of these compounds to generate nitric oxide in living organisms.

Conclusions

To sum up, the metabolic transformations involve various reactions typical of organic compounds.

A huge number of enzymes that function in living organisms can simultaneously metabolize both endogenous compounds and, without the strict substrate specificity, exogenous compounds, for example, drugs. It is obvious that endogenous and exogenous compounds can compete for the enzymes. The latter compounds can cause (and, most likely, actually cause) many pharmacological or toxic effects of the drugs.

Radical and dramatic structural differences between endogenous and exogenous compounds are virtually absent due to which systems of the body responsible for metabolic transformations can response to the appearance of new, previously unknown compounds and new types of substances. In the course of evolution, the enzymatic systems, which catalyze reactions with the involvement of endogenous substances, were apparently improved, resulting in a decrease in the substrate specificity, thus allowing the involvement of new series of chemical compounds.

From the present review, it is evident that only a combination of biological and chemical approaches can provide better insight into the observed phenomena, help in drawing more rigorous conclusions, and ensure the predictability of the development of the most promising fields of this science. It is necessary to perform these investigations jointly by chemists, pharmacologists, and biochemists. Unfortunately, chemists and physical chemists little participate in the discussion of biological and medical problems and it is necessary to stimulate the collaboration of chemists and biologists in studies of the most important and sophisticated phenomena observed in studies of biological and, in particular, metabolic, transformations. This review will hopefully encourage this collaboration.

References

1. B. Testa, *Metabolism of Drugs and Other Xenobiotics, Biochemistry of Redox Reactions*, Eds B. Testa, J. Caldwell, London—San Diego—New York—Boston—Sydney—Tokyo—Toronto, Academic Press, 1995, 471 p.
2. B. Testa, J. M. Mayer, *Hydrolysis in Drug and Prodrug Metabolism*, Verlag Helvetica Chimica Acta, Zurich—Switzerland, 2003, 780 p.
3. V. G. Granik, *Metabolizm endogennykh soedinenii* [*Metabolism of Endogenous Compounds*], Vuzovskaya kniga, Moscow, 2005, 528 pp. (in Russian).
4. V. G. Granik, *Metabolizm ekzogennykh soedinenii* [*Metabolism of Exogenous Compounds*], Vuzovskaya kniga, Moscow, 2006, 528 pp. (in Russian).
5. S. Primrose, R. Twyman, *Genomics. Application in Human Biology*, Oxford, Blackwell Publishing, Blackwell Science, 2004.
6. S. B. Seredenin, *Lektsii po farmakogenetike* [*Lectures in Pharmacogenetics*], Meditsinskoe informatsionnoe agenstvo, Moscow, 2004, 304 pp. (in Russian).
7. J. Uetrecht, *Current Drug Metabolism*, 2000, **1**, 133.
8. D. M. Ziegler, *Annu. Rev. Pharmacol. Toxicol.*, 1993, **33**, 179.
9. G. M. Wall, J. K. Baker, *J. Med. Chem.*, 1989, **32**, p. 134010.
10. M. C. Walker, J. T. Hazzard, G. Tollin, D. E. Edmondson, *Biochemistry*, 1991, **30**, 5.
11. S. Vickers, S. L. Polsky, *Current Drug Metabolism*, 2000, **1**, 387.
12. T. Baba, H. Yamada, K. Oguri, H. Yoshimura, *Biochem. Pharmacol.*, 1987, **36**, 4171.
13. K. Yuno, H. Yamada, K. Oguri, H. Yoshimura, *Biochem. Pharmacol.*, 1990, **40**, 2380.
14. G. Lin, K.-W. Chu, L. A. Damani, E. M. Hawes, *J. Pharm. Biomed. Anal.*, 1996, **14**, 727.
15. P. K. Arora, N. J. Riachi, S. I. Harik, L. M. Sayre, *Biochem. Biophys. Res. Commun.*, 1988, **152**, 1339.
16. V. G. Granik, *Osnovy meditsinskoi khimii* [*Fundamentals of Medical Chemistry*], Vuzovskaya kniga, Moscow, 2001, 384 pp. (in Russian).
17. J. I. Usansky, L. A. Damani, *Drug Metab. Disposit*, 1992, **20**, 64.
18. M. A. Schwartz, T. H. Williams, S. J. Kolis, E. Postma, G. J. Sasso, *Drug Metab. Disposit*, 1978, **6**, 647.
19. P. R. Ortiz de Montellano, *Trends Pharmacol. Sci.*, 1989, **10**, 354.
20. F. P. Guengorich, R. H. Bocher, *J. Biol. Chem.*, 1988, **263**, 8168.
21. F. P. Guengorich, *Chem. Res. Toxicol.*, 1990, **3**, 21.
22. J. W. Gorrod, *Biological Oxidation of Nitrogen in Organic Molecules. Chemistry, Toxicology and Pharmacology*, Eds J. W. Gorrod, L. A. Damani, Horwood, Chichester, 1988, pp. 219—230.
23. B. Clement, J. L. Boucher, D. Monsuy, *Biochem. Pharmacol.*, 1999, **58**, 439.
24. B. Clement, M.-H. Shultze-Mosgau, H. Wohlers, F. Jung, in *Enzymology and Biochemistry*, Eds M. Feelish, R. Busse, S. Moncada, Portland Press Ltd, London, 1994, 15.

25. D. Mansuy, J. L. Boucher, B. Clement, *Biochimie*, 1975, 7761.
26. (a) R. Jauch, E. Griesser, G. Oesterhelt, *Arzneim.-Forsch. (Drug Res.)*, 1980, **30**, 60; (b) M. K. P. Hoag, M. Schmidt-Peets, L. Lampen, A. Trevor, N. Castagnoli, *Chem. Research Toxicol.*, 1988, **1**, 128; (c) V. Z. Gorkin, *Aminooksidazy i ikh znachenie v meditsine* [*Amine Oxidases and Their Role in Medicine*], Meditsina, Moscow, 1981, 356 pp. (in Russian).
27. M. H. Bickel, *Pharmacol. Rev.*, 1969, **21**, 325.
28. T. Hauashi, M. Amino, Y. Ikoma, *Xenobiotica*, 1983, **13**, 461.
29. R. F. Struck, M. C. Kirk, M. H. Witt, W. R. Laster, *Biomed. Mass Spectrom.*, 1975, **2**, 46.
30. G. R. Murray, G. M. Whiffin, R. A. Franklin, J. A. Henry, *Xenobiotica*, 1989, **19**, 669.
31. R. N. Pandey, A. P. Armstrong, P. F. Hollenberg, *Biochem. Pharmacol.*, 1989, **38**, 2181.
32. A. P. Kulkarni, *Current Pharm. Design*, 2001, **7**, 833.
33. V. G. Kukes, *Metabolizm lekarstvennykh sredstv: kliniko-farmakologicheskie aspekty* [*Metabolism of Drugs: Clinical and Pharmacological Aspects*], Reafarm, Moscow, 2004, 144 pp. (in Russian).
34. C. G. Hover, A. P. Kulkarni, *Placenta*, 2000, **21**, 646.
35. S. Kanekal, J. P. Kehrer, *Drug Metab. Disposit*, 1993, **21**, 37.
36. K. Matsue, M. Mishima, Y. Nagai, T. Yuzuriha, T. Yoshimura, *Drug. Metab. Disposit*, 1999, **27**, 1406.
37. A. Assandri, A. Perazzi, P. Ferrari, E. Martinelli, A. Ripatonti, G. Tarzia, G. Tuan, *Naunyn Schmiedebergs, Arch. Pharmacol.*, 1985, **328**, 341.
38. S. Moncada, R. M. S. Palmer, E. A. Higgs, *Pharmacol. Rev.*, 1991, **43**, 109.
39. M. A. Marletta, *J. Med. Chem.*, 1994, **37**, 1899.
40. O. W. Griffith, D. J. Stuehr, *Annu. Rev. Physiol.*, 1995, **57**, 707.
41. V. G. Granik, S. Yu. Ryabova, N. B. Grigor´ev, *Usp. Khim.*, 1997, **66**, 792 [*Russ. Chem. Rev.* (*Engl. Transl.*), 1997, **66**].
42. A. F. Vanin, *Biokhimiya*, 1998, **63**, 924 [*Biochem. Moscow* (*Engl. Transl.*), 1998, **63**].
43. I. S. Severina, *Biokhimiya*, 1998, **63**, 939 [*Biochem. Moscow* (*Engl. Transl.*), 1998, **63**].
44. E. B. Men´shikova, N. K. Zenkov, V. P. Reutov, *Biokhimiya*, 2000, **65**, 485 [*Biochem. Moscow* (*Engl. Transl.*), 2000, **65**].
45. *Nitric Oxide, Basic Research and Clinical Application*, Eds R. J. Gryglewsky, P. Minuz, IOS Press, Amsterdam—Berlin—Oxford—Tokyo—Washington, DC, 2001.
46. D. Voet, J. G. Voet, *Biochemistry*, 2nd ed., John Wiley and Sons Inc., Chichester—Bricbana—Toronto—Singapore—New York, 1995, p. 1290.
47. F. Murad, *Neurotransmissions*, 1994, **X** (2), 1.
48. H. Al-Sa Doni, A. Ferro, *Clinical Science*, 2000, **98**, 507.
49. M. Feelish, *J. Cardiovascular. Pharm.*, 1991, **17** (Suppl. 3), 25.
50. V. P. Reutov, E. G. Sorokina, V. E. Okhotin, N. S. Kositsyn, *Tsiklicheskie prevrashcheniya oksida azota v organizme mlekopitayushchikh* [*Cyclic Transformations of Nitric Oxide in Mammals*], Nauka, Mir, 1998, 156 pp. (in Russian).
51. G. Wu, S. M. Morris, *Biochem. J.*, 1998, **336**, 1.
52. V. G. Granik, N. B. Grigor´ev, *Oksid azota* [*Nitric Oxide*], Vuzovskaya kniga, Moscow, 2004, 359 pp. (in Russian).
53. A. F. Vanin, I. V. Malenkova, V. A. Serezhenkov, *Nitric Oxide: Biol. Chem.*, 1997, **1**, 191.
54. V. A. Makarov, A. V. Danilov, V. I. Levina, N. B. Grigor´ev, M. Shmidke, A. Shtel´tsner, V. G. Granik, *Vopr. Biol. Med. Farm. Khim.* [*Probl. Biol. Med Pharm. Chem.*], 2002, No. 6, 45 (in Russian).
55. K. Schonafinger, *Farmaco*, 1999, **54**, 316.
56. S. Tanayama, Y. Nakai, T. Fujujta, Z. Suzuoki, *Xenobiotica*, 1974, **4**, 175.
57. H. Bohn, K. J. Schonafinger, *Cardiovasc. Pharmacol.*, 1989, **14** (Suppl. 11), 6.
58. T. Ullrich, S. Oberle, A. Abate, H. Schroeder, *FEBS Lett.*, 1997, **406**, 66.
59. V. I. Levina, N. B. Grigor´ev, V. G. Granik, *Khim. Geterotsikl. Soedin.*, 2004, 604 [*Chem. Heterocycl. Compd.* (*Engl. Transl.*), 2004].
60. N. V. Pyatakova, O. G. Busygina, I. S. Severina, N. B. Grigor´ev, V. G. Granik, *Khim. Geterotsikl. Soedin.*, 2002, 1665 [*Chem. Heterocycl. Compd.* (*Engl. Transl.*), 2002].
61. E. Yu. Khmel´nitskaya, V. I. Levina, L. A. Trukhacheva, N. B. Grigor´ev, V. N. Kalinin, I. A. Cherepanov, S. N. Lebedev, V. G. Granik, *Izv. Akad. Nauk, Ser. Khim.*, 2004, 2725 [*Russ. Chem. Bull., Int. Ed.*, 2004, **53**, 2840].
62. N. B. Grigor´ev, G. V. Chechekin, A. P. Arzamaztsev, V. I. Levina, V. G. Granik, *Khim. Geterotsikl. Soedin.*, 1999, 902 [*Chem. Heterocycl. Compd.* (*Engl. Transl.*), 1999].
63. N. F. Russo, M. Tomasz, M. Muller, R. Lipman, *Mol. Pharmacol.*, 1977, **13**, 872.
64. R. J. Knox, R. C. Knight, D. I. Edwards, *Biochem. Pharmacol.*, 1983, **32**, 2149.
65. J. Jin-Young, D. E. Berg, *Antimicrob. Agents Chemother.*, 2000, **44**, 3127.
66. R. P. Mason, P. D. Josephy, *J. Inorg. Biochem.*, 1985, **24**, 161.
67. K. Hofmann, in *The Chemistry of Heterocycl. Compounds — Imidazole and its derivatives*, New York, Interscience, 1953, 135.
68. A. F. Cockerill, R. C. Harden, D. N. B. Mallen, *J. Chem. Soc., Perkin Trans. 2*, 1972, 1428.
69. N. B. Grigor´ev, V. I. Levina, O. V. Azizov, N. V. Pyatakova, A. P. Arzamastsev, I. S. Severina, V. A. Parshin, V. G. Granik, *Vopr. Biol. Med. Farm. Khim.* [*Probl. Biol. Med Pharm. Chem.*], 2002, No 4, 10 (in Russian).
70. V. I. Levina, O. V. Azizov, N. V. Pyatakova, A. P. Arzamastsev, I. S. Severina, N. B. Grigor´ev, V. G. Granik, *Vopr. Biol. Med. Farm. Khim.* [*Probl. Biol. Med Pharm. Chem.*], 2002, No. 4, 6 (in Russian).
71. M. D. Mashkovskii, *Lekarstvennye sredstva. Posobie dlya vrachei* [*Drugs. Physician's Handbook*], 13th ed., Kharkov, Torgsin, 1997, **1**, 2 (in Russian).
72. C. Viode, N. Bettache, N. Cenas, *Biochem. Pharm.*, 1999, **57**, 549.
73. J. P. Salo, H. Salomies, *J. Pharm. Biomed. Anal.,* 1996, **14**, 1267.
74. L. F. Chasseaud, K. Henrick, R. W. Matthews, P. W. Scott, S. G. Wood, *J. Chem. Soc., Chem. Commun.*, 1984, 491.
75. L. A. Trukhacheva, N. B. Grigor´ev, A. P. Arzamastsev, I. L. Dalinger, I. A. Vatsadze, G. P. Popova, S. A. Shevelev, V. G. Granik, *Izv. Akad. Nauk, Ser. Khim.*, 2005, 2719 [*Russ. Chem. Bull., Int. Ed.*, 2005, **54**, 2813].
76. O. B. Ryabova, E. Yu. Khmel´nitskaya, V. A. Makarov, L. M. Alekseeva, N. B. Grigor´ev, V. G. Granik, *Izv. Akad. Nauk, Ser. Khim.*, 2005, 2777 [*Russ. Chem. Bull., Int. Ed.*, 2005, **54**, 2873].
77. O. B. Ryabova, V. A. Makarov, V. V. Chernyshev, V. G. Granik, *Izv. Akad. Nauk, Ser. Khim.*, 2004, 846 [*Russ. Chem. Bull., Int. Ed.*, 2004, **53**, 882].

Received June 17, 2009;
in revised form September 30, 2009